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CARCINOMA CELLS IN THORACIC AND IN ABDOMINAL FLUIDS

MONROE J. SCHLESINGER, PH.D., M.D.

BOSTON

For over fifty years there have appeared reports on the search for and the recognition of carcinoma cells in fluids aspirated from the thoracic or the peritoneal cavity. However, few clinicians think of requesting such examinations, because many of the pathologists associated with them consider it improbable that such a search will consistently yield conclusive diagnostic results. The favorable opinion of such examinations presented here is based on the findings in 100 cases in which carcinoma was being considered in the differential diagnosis and in which such an examination was made by a uniform technic.

Quincke [1] in 1875 was probably the first to see, describe and report carcinoma cells in smears from pleural and abdominal fluids. Since then there has appeared a long series of reports [2] of such findings in stained smears, always with a changing and presumably improving technic. Many of these reports, although based on observations of smears from but one or a few fluids, present elaborate discussions and numerous illustrations of the distinguishing minute peculiarities of the individual carcinoma cell. In the comment on such smears, emphasis is placed on such characteristics as the presence of mitotic figures, of clumps of cells

From the Pathology Laboratory, Beth Israel Hospital, and the Department of Pathology, Harvard Medical School.

1. Quincke, H.: Deutsches Arch. f. klin. Med. **16**:121, 1875; **30**:580, 1882.

2. Neelsen, F.: Deutsches Arch. f. klin. Med. **31**:375, 1882. Dock, G.: Am. J. M. Sc. **113**:655, 1897. Warthin, A. S.: M. News **71**:489, 1897. Gulland, G. L.: Scotch M. & S. J. **10**:490, 1902. Labbé; Delillie, A., and Aguimet: Bull. et mém. Soc. anat. de Paris **4**:507, 1902. Gwyn, N. B.: Am. J. M. Sc. **127**:592, 1904. Miller, J. L.: Am. Med. **8**:835, 1904. Turton, E.: Practitioner **74**:497, 1905. Ross, E. A.: Tr. Path. Soc. London **57**:361, 1905-06. Vickery, H. F., and Richardson, O.: Boston M. & S. J. **157**:824, 1907. Facchini, G.: Policlinico (sez. prat.) **15**:977, 1908. Marini, G., and Fiorini, M.: Riforma med. **24**:1, 1908. Widal, F., and Abrami, P.: Bull. et mém. Soc. méd. d. hôp. de Paris **25**:335, 1908. Warren, L. F.: Arch. Int. Med. **8**:648, 1911. Judd, C. C. W.: Am. J. M. Sc. **153**:717, 1917. Feinblatt, H. M.: M. J. & Rec. **121**:609, 1925. Quensel, W.: Acta med. Scandinav. **68**:427 and 458, 1928. Karp, H.: Ztschr. f. Krebsforsch. **36**: 579, 1932. McDonald, J. R., and Broders, A. C.: Arch. Path. **27**:53, 1939.

or of vacuolated cells. Variations in the size and shape of the nuclei are considered important. Some lay special emphasis on the nucleus-cytoplasm or the nucleus-nucleolus ratio.

At first my co-workers and I also utilized direct smears and tried various staining methods, without much success. In some of these smears we found a few cells strongly suggesting carcinoma cells. In the paraffin sections made from the sedimented cells from these same fluids there were innumerable regions easily recognizable as neoplastic. Most pathologists still agree with Borst [3] that individual neoplastic cells, even malignant ones, have no absolute specific morphologic characteristics such as (1) degree of dedifferentiation, (2) irregularities of such cytoplasmic details as granules, mitochondria or golgi network, or (3) nuclear changes, such as hyperchromatism or hypochromatism or abnormality of centrosomes or of nucleoli. Cytologic identification of the individual carcinoma cell is for the present not uniformly possible.

Nevertheless, with all these limitations in mind, it is usually not difficult to identify as neoplastic a comparatively small group of epithelial cells in tissue sections. This recognition is based as often on the relation of these epithelial cells to one another and to the surrounding structures as on the minute characteristics of the cells themselves. It is very difficult to preserve this structural picture in smears; hence the decision there must rest almost entirely on cytologic details. The method of centrifuging these fluids and embedding and sectioning the sedimented cells permits the study of whatever histoid characteristics are present, and for this reason alone it is a more reliable technic.

Scattered instances are recorded [4] in which a small bit of tissue was found floating in the aspirated fluid. This bit of tissue, separately embedded and sectioned, served best for the diagnosis. However, such accidental findings cannot be relied on for a diagnostic method, and as early as 1901 Josefson [5] advocated centrifuging these fluids and sectioning the sedimented cells. Even before this, in 1895, Bahrenberg [6] published a similar technic in the little read *Cleveland Medical Gazette*. In 1917 Mandlebaum,[7] of the Mount Sinai Hospital, New York,

3. Borst, M.: Die Lehre von den Geschwülsten, Wiesbaden, J. F. Bergmann, 1902.

4. Prentiss, D. W.: Tr. A. Am. Physicians **8**:191, 1893. Rieder, H.: Deutsches Arch. f. klin. Med. **64**:544, 1895. Steiner, W. R.: Bull. Johns Hopkins Hosp. **12**:310, 1901. Steele, J. D., and Girvin, H., Jr.: Proc. Path. Soc. Philadelphia **4**:164, 1901. Nattau-Larrier, M. L.: Compt. rend. Soc. de biol. **58**:709, 1905. Levesque, J.: Médecine **5**:958, 1924. Bock, E.: Klin. Wchnschr. **4**:651, 1925.

5. Josefson, A.: Hygiea **63**:435, 1901; Ztschr. f. klin. Med. **82**:331, 1916; Acta med. Scandinav. **53**:770, 1921.

6. Bahrenberg, L. H. P.: Cleveland M. Gaz. **11**:274, 1895.

7. Mandlebaum, F. S.: J. Lab. & Clin. Med. **2**:580, 1917.

reported using this method, but it was not until 1928 that Zemansky [8] published his report of a series of 113 such examinations made in that institution. Meanwhile, Ellis,[9] of the Memorial Hospital, New York, in 1922 emphasized the value of a similar technic, and Foot [10] in 1937 again reviewed the material from that hospital. Also, Seecof and Boetsch [11] in 1924 reported on 93 cases studied by a similar method at the Montefiore Hosptial, New York.

TECHNIC

The technic followed is extremely simple. The fluid is centrifuged at high speed until the supernatant liquid is clear. This is most conveniently accomplished by centrifuging repeatedly in the same 50 cc. round bottom centrifuge tube, pouring off the cleared fluid and replacing it by fresh fluid to be centrifuged. Sufficient sediment is thus finally obtained as one mass at the bottom of the centrifuge tube. Even if the fluid has not been collected aseptically, its preservation in the ice box for twenty-four or thirty-six hours before centrifuging permits little effect on the neoplastic cells. Also, centrifuging for a considerable period at the highest speed obtainable does not damage the cells.

The sedimented mass obtained is then prepared for fixation. It is first transferred to a piece of filter paper as a small compact pile. This transfer is made either with a small spatula, the point of a scalpel or a small platinum loop, depending on the amount and consistency of the sediment. The sediment obtained is seldom insufficient for such transfer, especially the sediment from fluids containing neoplastic cells. Sometimes a fibrin clot forms in the fluid, and then there is obtained after centrifuging a voluminous jelly-like precipitate, which comes out of the tube en masse. In this clot are enmeshed all the cells of the fluid. Before fixing, some of the fluid that is also enmeshed in the clot is extracted by gently rolling the clot around on dry filter paper. The sediment or clot is fixed in at least 10 volumes of a neutral 1:10 dilution of solution of formaldehyde U. S. P. for eighteen to twenty-four hours, embedded in paraffin, sectioned at 8 to 10 microns and stained with hematoxylin and eosin. This method differs from the original method of Josefson,[5] who added alcohol to the fluid before centrifuging and thus obtained a more voluminous precipitate of coagulated protein. Ellis [9] mixed solution of formaldehyde instead of alcohol with the fluid. Both these procedures yield a flaky, nonadhesive precipitate, which is difficult to handle and section. It has not been found necessary or desirable to fix the precipitate directly in the centrifuge tube as practiced by Mandlebaum [7] and by Zemansky.[8]

PATHOLOGIC PICTURE

Graham,[12] who used a comparable technic on 50 such fluids, has presented the most recent extensive discussion of the appearance of carcinoma cells in sections of the sediment of effusions. Although we have encountered all the pictures he so well described, we have never felt it safe to base a diagnosis on the appearance of individual cells. In

8. Zemansky, A. P., Jr.: Am. J. M. Sc. **175**:489, 1928.
9. Ellis, E. B.: Bull. Internat. A. M. Museums **8**:126, 1922.
10. Foot, N. C.: Am. J. Path. **13**:1, 1937.
11. Seecof, D. P., and Boetsch, N.: Proc. New York Path. Soc. **24**:2, 1924.
12. Graham, G. S.: Am. J. Path. **9**:701, 1933.

every instance we have made the diagnosis of carcinoma only after finding a group of cells whose arrangement left no doubt as to their epithelial nature. The individual cells comprising the group were incontrovertibly separated by definite cell walls. The cells had a definite polygonal shape, often cuboid to high columnar. The group of cells having these characteristics may have been small in number, sometimes comprising only 4 to 6 cells. These few cells, however, were definitely arranged in the form of an acinus or a part of an acinus. In such an arrangement the nuclei of the cells exhibited definite polarity, all being located in the same position relative to the lumen or to the periphery of the acinus. These criteria for the recognition of carcinoma cells can be amplified best by discussing a few illustrative cases with their accompanying photomicrographs.

CASE 1.—*Carcinoma of the Ovary* (fig. 1).—A 46 year old woman had swelling of the abdomen, dyspnea, anorexia and some loss of weight for three weeks. Examination showed only marked ascites and edema of the sacrum and ankles. The diagnosis was held in abeyance until after a paracentesis had been performed. Then a mass in the pelvis could be felt, and carcinoma of the ovary was suspected.

Sections.—The initial few routine sections from the sedimented cells of the abdominal fluid were inconclusive in that, though a few suspicious clumps of cells were encountered, there was nothing of absolute diagnostic value. In figure 1 (*1*) are two clumps of cells, obviously different from the isolated cells sprinkled about them and resembling epithelial cells to the point of arousing a suspicion of carcinoma. The upper, larger clump, *A,* is the least epithelial-like of the two, but careful focusing on these cells showed definite cell walls between them in many places. A sharply defined, limiting cell wall between two contiguous cells is one of the most useful criteria in recognizing epithelial cells. The lower, smaller group, *B,* composed of only 4 cells, is more definitely epithelial, for in addition to sharply defined cell walls, seen on focusing, the cells show some polarity. Aiming to examine more closely this lower clump of cells, we cut additional serial sections from this paraffin block. Only rarely has it been necessary to resort to serial sections. However, originally we generally prepared a short ribbon of sections from each block, cutting, mounting and staining a long enough strip to cover two or three microscopic slides. Thus, any suspected group of cells may be followed through for a short distance. In this particular instance, the smaller group, *B,* disappeared in the serial sections, but the larger group, *A,* took on a more characteristic appearance of epithelial cells. The next two sections shown in figure 1 (*2* and *3*) are from this series but are not consecutive. In these sections the polarity of the cells in question is more obvious, and a definite tendency to form an acinus is revealed. There is one definite mitotic figure shown at *C.* Although others have emphasized mitotic figures as of great diagnostic

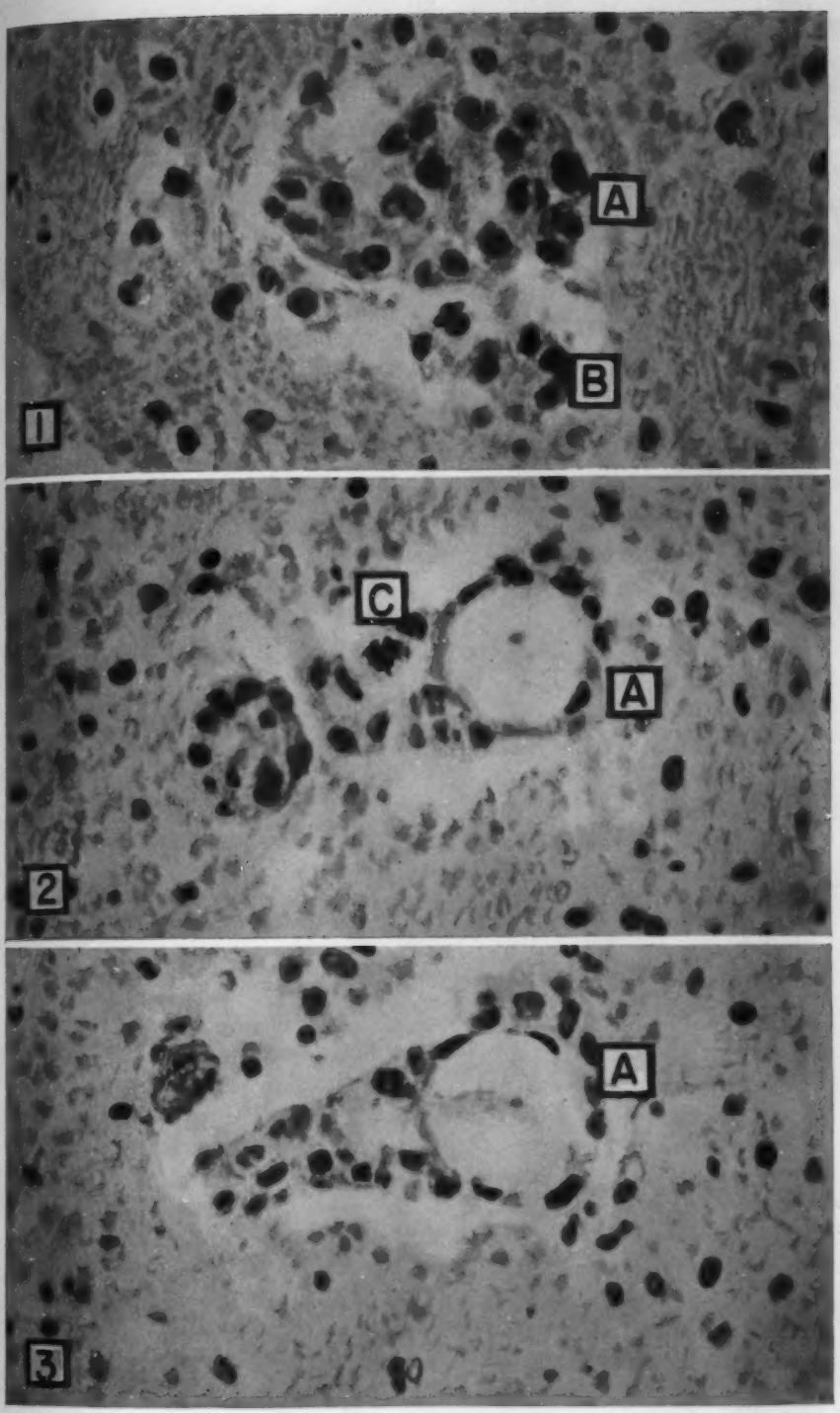


Fig. 1 (case 1).—*1*, *2* and *3*, serial sections of cellular sediment from abdominal fluid removed from a patient with carcinoma of the ovary; × 400.

significance in recognizing carcinoma cells, not much reliance can be placed on this finding. In many otherwise most convincing preparations no mitotic figures could be found.

Comment.—Following the confirmation of the clinical diagnosis of carcinoma by the sections, a laparotomy was performed, and an inoperable carcinoma of the ovary with peritoneal metastases was found.

CASE 2.—*Carcinoma of the Ovary* (fig. 2 [*4*]).—For two months a 60 year old woman had noticed increase in the size of the abdomen, increase in weight, loss of appetite and development of dyspnea. Examination revealed only ascites. Before tapping the differential diagnosis was cirrhosis of the liver, ovarian cyst or carcinoma. After paracentesis a pelvic mass was palpable. Sections from the sedimented cells of abdominal fluid showed no neoplastic cells, however. A second tap was performed ten days later and the sedimented cells sectioned.

Sections.—In figure 2 (*4*), representing this second specimen of fluid, is a definite papillary structure, with the cells arranged about the periphery and demonstrating a special type of polarity. A central stromal core is suggested. In no other fluid have we found anything suggesting a stroma supporting clumps of neoplastic cells, although Graham reported this finding several times.

Comment.—The patient was discharged with a diagnosis of inoperable papillary carcinoma of the ovary. This was not confirmed by another, independent objective method, for no other confirmation of the picture shown was considered necessary. Later a metastasis developed at the site of the paracentesis.

CASE 3.—*Carcinoma of the Ovary* (fig. 2 [*5*]).—Six months previously the patient, a 49 year old woman, had been operated on for "pus kidney." For two months she had had rectal pain, and for several weeks diarrhea, with bloody stools. For two weeks her abdomen had been swollen. Roentgen examination on admission showed only calcified leiomyoma. The diagnosis on admission was probable carcinoma of the large bowel and splenomegaly. Paracentesis was done.

Sections.—The sections presented a solid mass of similar appearing cells, the majority of which were probably epithelial cells. From such areas as appear at *A* in figure 2 (*5*), representing these sections, a positive diagnosis of carcinoma could not be made. Similarly at the lower border on the right, at *B*, the cells immediately around what appears to be a large lumen are compressed, have no polarity and are not arranged as a true acinus. At the upper border are three masses of cells more definitely arranged like epithelial cells. The mass at *C* has only a suggestive arrangement. The right hand mass at *D*, however, more definitely resembles an acinus, but most of its cells are indistinct. The left hand mass, at *E*, is undoubtedly half of an acinus. In this fluid the tumor cells were so numerous that a suspicion of carcinoma was aroused from a smear stained with Wright's stain.

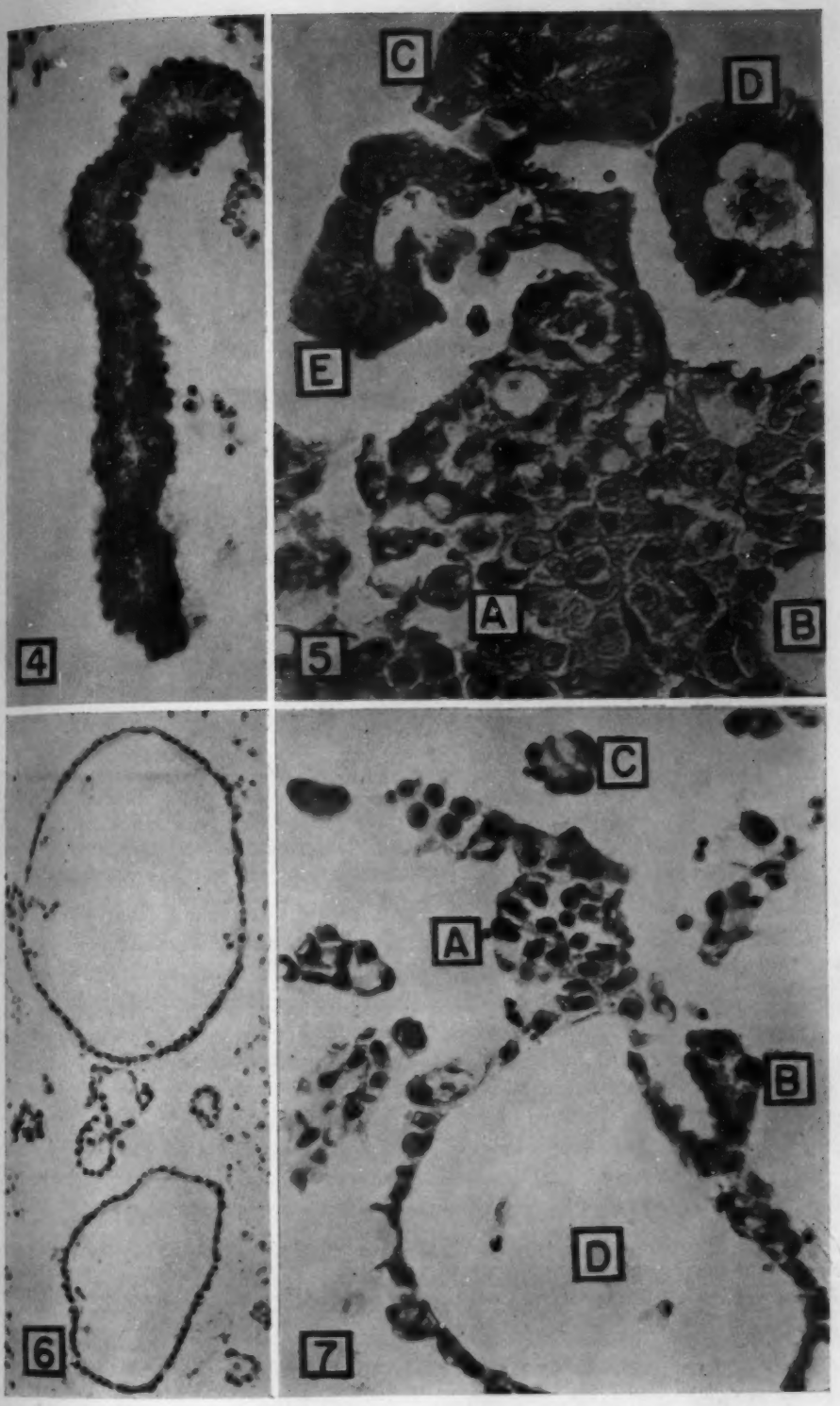


Fig. 2.—Sections of cellular sediment from abdominal fluid removed from patients with: (*4*) carcinoma of the ovary (case 2); × 200; (*5*) carcinoma of the ovary (case 3); × 200; (*6*) carcinoma of the ovary (case 4); × 100; (*7*) carcinoma of the uterus (case 5); × 200.

Comment.—In this patient the sections from the sedimented cells of the fluid established the diagnosis, although the primary site of the carcinoma could not be localized. Usually it is not possible to identify the primary source of the carcinoma cells recognized in these effusions. In this patient an exploratory laparotomy disclosed carcinoma of the ovary with peritoneal metastases.

CASE 4.—*Carcinoma of the Ovary* (fig. 2 [*6*]).—A 48 year old woman had complained for four months of distention, pain and tenderness of the abdomen and of loss of weight. She was considered to have quiescent pulmonary tuberculosis with probable tuberculous peritonitis. An exploratory operation showed carcinoma of the ovary with multiple peritoneal metastases. Roentgen treatments were instituted. Later there was recurrence of the ascites, and on three occasions fluid was removed and the sedimented cells sectioned.

Sections.—On all three occasions the sections showed numerous well formed acini composed of cuboid epithelial cells. Several of these acini are shown in figure 2 (*6*). These present no difficulty in diagnosis.

Comment.—Probably a preoperative paracentesis with sectioning of the sedimented cells of the fluid would have established the diagnosis and made the laparotomy unnecessary. At all events, the repeated finding of carcinoma cells in the aspirated fluid proved the ineffectiveness of the roentgen treatments.

CASE 5.—*Carcinoma of the Uterus* (fig. 2 [*7*]).—In a 60 year old woman curettage four months before admission revealed carcinoma of the fundus of the uterus. While she was under treatment for this, her abdomen got larger, and she lost weight. The ascites was relieved by tapping, and the sedimented cells of two specimens of the fluid, removed thirty-five days apart, were sectioned.

Sections.—Sections representing the first removal of fluid showed many masses of vacuolated epithelial-like cells, most of which were not definite enough to permit an absolute diagnosis of carcinoma. In figure 2 (*7*), such a mass is seen at *A*, just above the large acinus-like group. However, at *B*, just to the right of the large acinus, is a cluster in which the left hand four cells show the polarity characteristic of epithelial cells. At the extreme top, at *C*, is another group of vacuolated cells arranged as an acinus. The arrangement seen in the large oval acinus, *D*, is never taken except by epithelial cells. The vacuolation of the cells confuses what would otherwise be a picture very easy to interpret. The second examination of fluid showed a few similar groups of cells.

Comment.—In this case, sections of the sedimented cells of the fluid confirmed the suspicion that the carcinoma of the uterus had already spread to the peritoneum.

CASE 6.—*Carcinoma in the Lung* (fig. 3 [*8*]).—A 66 year old woman had pain in the lower region of the right side of the chest and loss of weight for one year. Eight days before she was admitted to the hospital, a slight cough and dyspnea developed, with increase in severity of this pain. She was cyanotic and had fluid

in her chest. Thoracentesis yielded 1,700 cc. of fluid and relieved the pain, dyspnea and cyanosis. A roentgenogram made after this tapping revealed definite carcinoma of the lung.

Sections.—The sections of the sedimented cells of the fluid showed innumerable nests of undoubted epithelial cells, everywhere exhibiting polarity and acinar formation, and the diagnosis of carcinoma was made without difficulty. At *A* in figure 3 (*8*) is seen the confusing vacuolation present in some of these cells. Some workers have emphasized this vacuolation as a characteristic of epithelial cells, but it is by no means a constant feature.

Comment.—In this instance the sections were merely confirmatory of a finally definite clinical diagnosis, but they served to clinch that diagnosis with a biopsy.

CASE 7.—*Carcinoma in the Lung* (fig. 3 [*9*]).—A 33 year old woman for two months had increasing dyspnea and dry cough. Signs of fluid were found over the entire left side of the thorax, and the differential diagnosis was tuberculosis, pleurisy or carcinoma of the lung. The chest was tapped, and part of the fluid was injected into a guinea pig, and sections were made from the sedimented cells of the rest. Roentgenograms taken after tapping revealed definite carcinoma at the base of the left lung.

Sections.—The sections showed definite carcinoma, but relatively few distinct acini were found. In figure 3 (*9*), in most places the groups of cells only suggest epithelial cell acini. Part of one of the two clumps at *A*, however, is definitely one half of an acinus composed of high columnar cells. In each of the other clumps, at *B* and *C*, at some place about their circumference a few cells present a definite tendency toward polarity.

Comment.—Here again the fluid made possible a biopsy of an otherwise inaccessible growth and established the diagnosis.

CASE 8.—*Carcinoma in the Lung* (fig. 3 [*10*]).—A 69 year old woman had vague pains in the left side of the chest for two years. Shortly before her admission to the hospital flaccid paralysis of the left side developed which had been preceded by pain in the left foot of increasing severity. On admission she had signs of a lesion of the spinal cord and of fluid in the thoracic cavity on the right, and the diagnosis of carcinoma of the lung with metastases to the spinal cord was made. The chest was tapped, and the sedimented cells of the fluid sectioned, but no carcinoma cells were found. Ten days later the cell mass from fluid obtained by another tap was sectioned.

Sections.—Only a few groups of undoubted neoplastic cells were present, but fortunately a group of cells as conclusive as those depicted in figure 3 (*10*) was found. In this group there are (1) acinar formation, (2) polarity, (3) sharply defined cell membranes and (4) a mitotic figure.

Comment.—Repeated roentgenograms, taken both before and after both thoracenteses, were never conclusive, and the diagnosis of a neo-

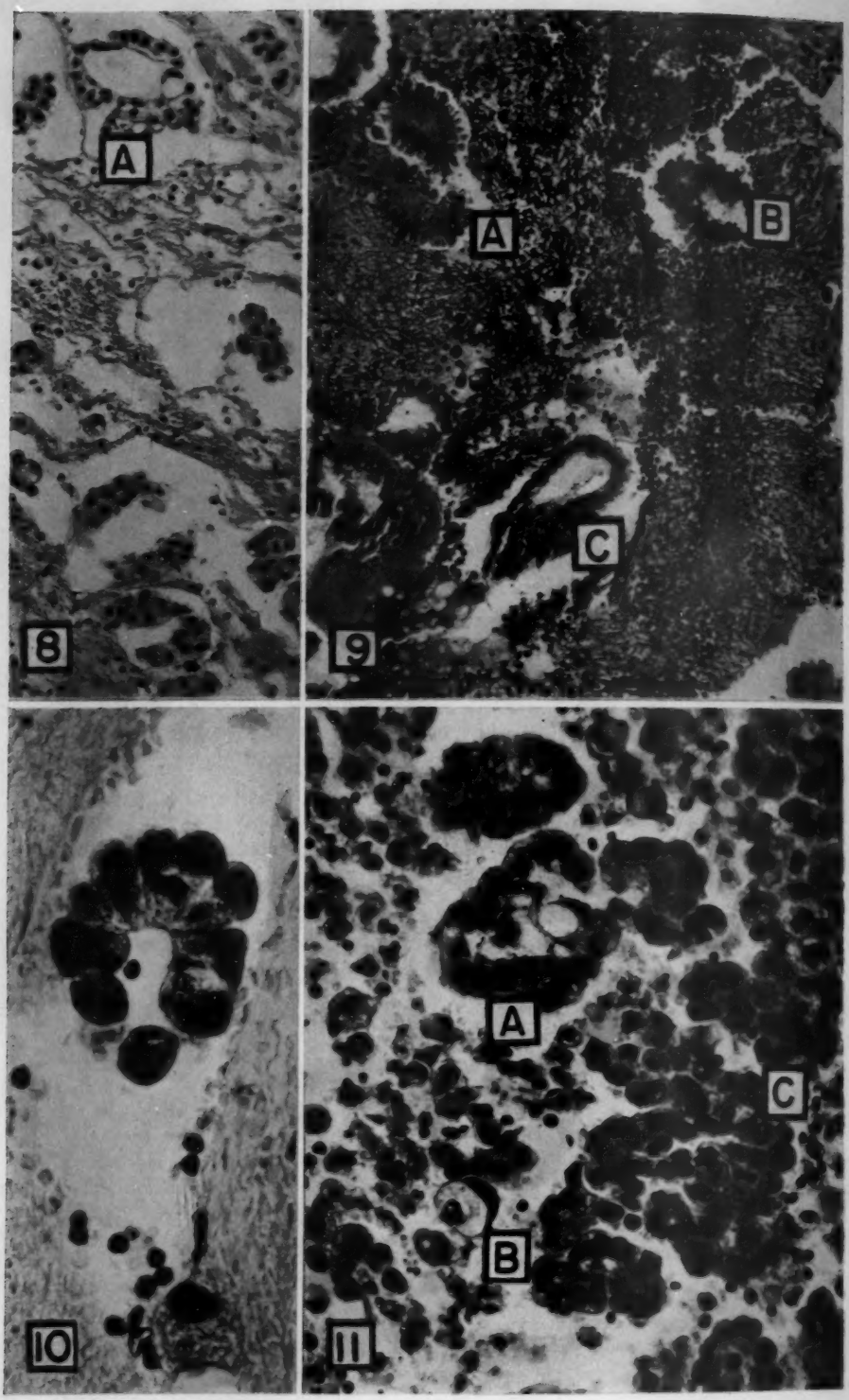


Fig. 3.—Sections of cellular sediment from thoracic fluid removed from patients with: (*8*) carcinoma in the lung (case 6); × 100; (*9*) carcinoma in the lung (case 7); × 100; (*10*) carcinoma in the lung (case 8); × 400; (*11*) carcinoma in the lung (case 9); × 200.

plasm was made entirely on clinical evidence. The sections of the sedimented cells from the fluid were the only objective confirmatory evidence, but they were unequivocal.

CASE 9.—*Carcinoma in the Lung* (fig. 3 [*11*]).—For three months a 64 year old man had had pain in the chest, cough, progressive weakness and loss of appetite. He had lost 18 pounds (8 Kg.) of weight in three weeks. He showed only clubbed fingers and much fluid in the left side of the chest. The differential diagnosis lay between tuberculosis with effusion and carcinoma. The chest was tapped two days in succession; part of the fluid was cultured, part was injected into guinea pigs, and cellular sediment of the fluid was sectioned on both occasions. The cultures showed no growth of tubercle bacilli and the results of the injections were negative.

Sections.—On both occasions the sections of the cellular sediment of the fluid showed numerous masses of neoplastic cells. Many acini, more definite than those shown in figure 3 (*11*), were seen. However, the clump at *A* is clearly enough composed of epithelial cells to establish the diagnosis of carcinoma. The field selected for illustration demonstrates the large number of isolated, less easily recognizable cells between the cell clumps. Many of these separate cells resemble the unquestionable epithelial cells; many are monocytes; many are of doubtful origin. At *B* is a cell phagocytosing a vacuolated cell. Five mitotic figures are shown, four in the lower right hand clump of cells at *C* and one in the clump at *A*.

Comment.—In this case a roentgenogram made after tapping confirmed the diagnosis of carcinoma of the lung, but the examination of the sections of the cellular sediment from the fluid removed on two occasions served very conveniently as a biopsy to establish the diagnosis.

SUMMARY OF RESULTS

Over a period of ten years this method of diagnosis, with use of the technic discussed and of the criteria for recognition of carcinoma cells, has been resorted to in connection with the examination of 145 samples of fluid withdrawn from 100 selected patients. For all these patients the diagnosis of carcinoma was being considered at the time the fluid was withdrawn, and it was hoped that a search for neoplastic cells might be of diagnostic aid. A much larger number of such effusions was withdrawn but not examined by this technic because the presence of carcinoma was either obvious or not suspected. The 145 samples of fluid selected for this type of examination comprised 76 thoracic and 69 abdominal fluids.

As indicated in table 1, some form of carcinoma was the diagnosis at discharge for 60 of these 100 patients and no neoplasm for the other 40. Sections of the sediments from the fluids showed neoplastic cells in 35, or 58.3 per cent, of the 60 cases of carcinoma. The highest incidence of carcinoma cells (100 per cent) was in the 10 cases of carcinoma of the ovary, as would be expected from the well known tendency of this

neoplasm toward peritoneal implantation. However, in 50 per cent or more of the cases of carcinoma in the lung or in the gastrointestinal tract the fluids contained such recognizable neoplastic elements. In only 1 of the 5 cases of metastatic carcinoma of the breast was the condition thus diagnosable, however. A diagnosis was made by this means in a single instance each of carcinoma of the uterus, of carcinoma of the pancreas and of malignant melanoma.

At the time of writing 21 of the 60 patients with carcinoma are alive or cannot be traced. The other 39 died of carcinoma within three years. In only 12 cases was permission for an autopsy obtained, and these autopsies were confirmatory. However, in 19 other cases operation

TABLE 1.—*Incidence of Carcinoma Cells in Thoracic and Abdominal Fluids*

Clinical Diagnosis	Cases	Cases in Which Given Fluid Was Examined		Carcinoma Cells in Fluid			Cases in Which Confirmatory Examinations		Patients Dead at		Patients Alive or Lost
		Thoracic	Abdominal	Cases in Which Cells Were Present	Cases in Which Cells Were Absent	Percentage of Cases in Which Cells Were Present	Were Recorded	Were Not Made	1 Yr.	3 Yr.	
Carcinoma of ovary...	10	0	10	10	0	100	9	1	3	4	3
Carcinoma of lung.....	26	26	0	13	13	50	19	7	13	2	11
Carcinoma of stomach	7	0	7	4	3	57	7	0	4	0	3
Carcinoma of colon....	8	0	8	4	4	50	8	0	5	0	3
Carcinoma of breast...	5	1	4	1	4	20	4	1	4	0	1
Carcinoma of thyroid..	1	1	0	0	1	0	1	0	1	0	0
Malignant melanoma..	1	1	0	1	0	100	1	0	1	0	0
Carcinoma of uterus...	1	0	1	1	0	100	1	0	0	1	0
Carcinoma of pancreas	1	0	1	1	0	100	0	1	0	1	0
Cases of carcinoma..	60	29	31	35	25	58.3	50	10	31	8	21
Cardiac failure.....	16	13	3	0	16	0	5	11	4	4	8
Cirrhosis of liver.....	9	1	8	1	8	11.1	5	4	4	4	1
Abscess of lung.....	7	7	0	0	7	0	4	3	2	1	4
Tuberculosis.....	4	2	2	0	4	0	2	2	1	0	3
Abscess of liver.....	1	1	0	0	1	0	1	0	1	0	0
Cyst of liver.....	1	1	0	0	1	0	1	0	1	0	0
Splenic infarct.....	1	1	0	0	1	0	1	0	1	0	0
Portal thrombosis.....	1	1	0	0	1	0	1	0	1	0	0
Cases in which carcinoma was not present..	40	26	14	1	39	2.5	20	20	15	9	16

yielded a confirmatory biopsy. In another 19 cases confirmatory objective evidence of carcinoma was obtained by taking an appropriate roentgenogram. Thus, in all but 10 (16.7 per cent) of the 60 cases of carcinoma, the clinical diagnosis was confirmed by another, independent method, such as roentgen study, operation, biopsy or autopsy. In the closely controlled smaller group of 31 cases in which the diagnosis of carcinoma was proved by an independent study of sections of tissue, available because of necropsy or operation or both, there were 20 (64.5 per cent) in which a positive diagnosis was made on the sections of the cellular sediment from the fluid.

In only a single case of a condition not carcinoma, finally shown to be a case of cirrhosis of the liver, were carcinoma cells erroneously

reported as present in the fluid first removed but not in that removed on three subsequent occasions. This erroneous report was made early in the course of this study. In a recent review of the incorrectly interpreted sections it was felt that the same error might be made again by one inexperienced in examining sectioned sediments. Further experience has demonstrated that ascitic fluids from patients with cirrhosis of the liver very often contain groups of large cells which might initially be confused with neoplastic epithelium. More careful study of these cells with use of the rigid criteria discussed in foregoing paragraphs should correct this first impression.

In no other case in which carcinoma was absent was such a diagnosis made on the sectioned sediment of the fluid, although 53 fluids from 40 noncarcinomatous patients were examined. It is important that the criteria and standards finally adopted for any laboratory procedure should be such that the number of erroneous positive reports is reduced to an absolute minimum. There is a definite tendency to place undue emphasis on such presumably objective positive findings. The physician in charge of a patient is less apt to be misled by an occasional erroneous negative laboratory report. If he is still in doubt, he may wish to repeat such an examination on a later occasion.

An interesting incidental observation was made that 46 of these 100 patients with ascites or hydrothorax survived less than one year after the fluid had accumulated, and 63 were known to be dead in less than three years. The poor prognosis for patients with fluids in the body cavities, from whatever cause, is thus amply confirmed.

REPEATED EXAMINATIONS

From 70 of this series of 100 patients, only fluid removed on a single occasion was available for study; from each of the other 30 patients fluid removed on two or more occasions was centrifuged and the sediment sectioned. Repeated examinations of this type were usually requested by the physician in charge of the patient because the observations on the first examination disagreed with his clinical diagnosis or because the diagnosis was still in doubt. In regard to these 30 patients the final clinical diagnosis was carcinoma for 21 and some other condition for 9.

In table 2 are presented the observations on the fluids from the 21 patients with carcinoma who had such examinations on two or more occasions. In only 4 of the 14 cases of carcinoma in which the result of the first examination of the fluid was negative were carcinoma cells found when the examination of the fluid was repeated. Contrariwise, in 7 cases in which originally the results were positive the results of 4 of the 11 repeat examinations were negative. However, in 2 of the latter cases, in other repeat examinations carcinoma cells were found. Repeat examinations are important and usually helpful when the diagnosis is still in doubt. Sometimes an unequivocal finding will be thus

obtained. At other times the most persistent repetition will not yield a conclusive result. There is a distinct limitation to the method, which must be recognized. As with most properly interpreted laboratory procedures, a negative result may be inconclusive even when repeated, whereas a positive finding should be regarded as of absolute diagnostic significance.

TABLE 2.—*Data on Repeated Examinations of Thoracic and Abdominal Fluids from Patients with Carcinoma*

Site of Carcinoma	Fluid Examined	Carcinoma Cells Revealed in Examinations of Fluid							Source of Independent Confirmation
		1st	Interval	2d	Interval	3d	Interval	4th	
Lung	Thoracic	0	6 days	0	.....	..	.....	..	
Lung	Thoracic	0	15 days	0	.....	..	.....	..	Roentgenogram
Lung	Thoracic	0	8 days	0	.....	..	.....	..	Necropsy
Colon	Abdominal	0	4½ mo.	0	.....	..	.....	..	
Colon	Abdominal	0	6 days	0	.....	..	.....	..	Operation and biopsy
Breast	Thoracic	0	5 days	0	.....	..	.....	..	Operation and biopsy
Stomach	Abdominal	0	14 days	0	10 days	0	.....	..	Roentgenogram and necropsy
Lung	Thoracic	0	8 days	0	51 days	..	.....	..	Roentgenogram
Lung	Thoracic	0	8 days	0	31 days	0	12 mo.	0	
Colon	Abdominal	0	6 days	0	2 yr. 60 days	0	8 mo.	0	Operation and biopsy
Ovary	Abdominal	0	10 days	+	.....	..	.....	..	Operation and biopsy
Lung	Thoracic	0	6 days	+	.....	..	.....	..	
Lung	Thoracic	0	5 days	+	.....	..	.....	..	
Lung	Thoracic	0	10 days	+	28 days	0	.....	..	
Ovary	Abdominal	+	7 days	0	.....	..	.....	..	Operation and biopsy
Lung	Thoracic	+	1 day	+	34 days	0	.....	..	Roentgenogram
Pancreas	Abdominal	+	9 days	0	10 days	0	7 days	+	
Uterus	Abdominal	+	35 days	+	.....	..	.....	..	Operation and biopsy
Ovary	Abdominal	+	8 days	+	.....	..	.....	..	Operation and biopsy
Lung	Thoracic	+	12 days	+	.....	..	.....	..	Operation and biopsy
Ovary	Abdominal	+	82 days	+	21 days	+	.....	..	Operation and biopsy; necropsy

COMMENT

In making a positive identification of epithelial cells in these sections the most useful characteristic is that the cells in question are oriented as if they had a certain degree of polarity. The arrangement usually is in the form of a whole or a part of an acinus, with or without a lumen. The cells in such a figure generally have their nuclei in the same relative position to the lumen or to the periphery of the acinus; i. e., the cells exhibit polarity. Also, when the cells are so arranged their boundaries are usually quite distinct, and the epithelial cells have a definite polygonal shape, often recognized only after careful focusing under oil immersion.

Occasionally the cells are actually definitely cuboid or columnar. The confusing monocytes and the desquamated serosal cells which usually are also present never have these characteristics. Isolated epithelial cells, however, are often indistinguishable from the latter cells.

The diagnosis of carcinoma by examination of thoracic and abdominal fluids is quite feasible without resorting to any really new principles. The method is not purely objective, however, and it has its limitations. In the final analysis the conclusion hinges on the opinion of the pathologist, on what he sees under the microscope. To be of value, this opinion must be supported by a certain amount of experience, but this experience is easily gained. Other common tests made on exudates, such as those taking account of specific gravity, total cell count, differential cell count, bacteriologic growth and other features, can be and usually are performed by technicians. Frequently the technicians work in a laboratory apart from the laboratories of pathology, where smears of such fluids are readily prepared, but not sections of the sedimented cells. The preparation and examination of sections of exudates for carcinoma cells must be made in the laboratory of pathology. In these sections the pathologist should recognize the microscopic masses of neoplastic tissue as readily as he does groups of tumor cells of similar dimensions in other, more familiar sections. He should not place too much dependence on a study of the minute characteristics of individual cells, no matter how numerous they are. His time will be better spent in a search for and study of groups of cells. He will then study many separate microscopic bits of tissue gathered together into a single section. This is quite analogous to the search in such fluids for pieces of tissue large enough to be seen with the naked eye. Many have commented on the ease of diagnosing a neoplasm by sectioning such relatively large bits of tissue.

SUMMARY AND CONCLUSIONS

The diagnosis of carcinoma can be made readily on thoracic and ascitic fluids. Consistent, reliable results can be obtained best by the technic of centrifugation of the fluid and fixation, embedding and sectioning of the sediment. The method of utilizing stained or unstained smears was found to be unreliable.

A definite positive diagnosis of carcinoma should be made on such sections only after finding groups of definite polygonal cells showing polarity, sharply distinct cell walls and acinar or pseudoacinar formation.

Such readily recognizable microscopic bits of tumor tissue are contained in about 60 per cent of the fluids accumulating in the cavities of the body as the result of carcinoma.

The cells of fluids accumulating in the cavities of the body from other causes never assume such a histoid appearance but may otherwise resemble carcinoma cells. If strict adherence to the aforementioned proper, rigid criteria is maintained, a false diagnosis of carcinoma will never be made on such a non-neoplastic fluid.

CEREBRAL LESIONS IN HYPOGLYCEMIA

III. EXPERIMENTAL INVESTIGATIONS

A. B. BAKER, M.D.
MINNEAPOLIS

The continued interest in hypoglycemia as a therapeutic agent has given a definite impetus to investigation of the resulting cerebral changes. The value of a thorough knowledge of the alterations produced in the brain by such treatment is of importance for an understanding not only of the mechanism of the beneficial results of this treatment but also of the dangers that might be involved because of the permanent damage and often continuing destruction of brain.

During the past few years there have appeared in the literature sporadic reports describing definite cerebral damage in persons suffering from hypoglycemia (Wolf and others;[1] Morsier and Mozer;[2] Salm;[3] Lindsay and others;[4] Moersch and Kernohan;[5] Kobler[6]). Brain changes of a similar nature have been recorded in the experimental animal. Schereschewsky and his co-workers[7] produced acute and chronic insulin poisoning in dogs. The brain tissue of these animals contained numerous old and new hemorrhages as well as many destroyed cortical nerve cells. These changes were scattered throughout the central nervous system but varied in intensity from animal to animal. Stief and Tokay[8] and Grayzel[9] described changes occurring almost exclusively in the nerve cells. Stief and Tokay produced their alterations in 4 rabbits and 4 dogs. The nerve cell changes varied from mild paling of the cell cytoplasm to complete chromatolysis, vacuolation,

From the Division of Nervous and Mental Diseases, University of Minnesota.

1. Wolf, A.; Hare, C. C., and Riggs, H.: Bull. Neurol. Inst. New York **3**:232, 1933.

2. Morsier, G., and Mozer, J. J.: Ann. de méd. **39**:474, 1936.

3. Salm, H.: München. med. Wchnschr. **84**:1046, 1937.

4. Lindsay, J. W.; Rice, E. C.; Selinger, M. A., and Mish, K. H.: Ann. Int. Med. **10**:1892, 1937.

5. Moersch, F. P., and Kernohan, J. W.: Arch. Neurol. & Psychiat. **39**:242, 1938.

6. Kobler, F.: Arch. f. Psychiat. **107**:688, 1938.

7. Schereschewsky, N. A.; Mogilnitzky, B. N., and Gorjaewa, A. W.: Endokrinologie **5**:204, 1929.

8. Stief, A., and Tokay, L.: Ztschr. f. d. ges. Neurol. u. Psychiat. **153**:561, 1935.

9. Grayzel, D. M.: Arch. Int. Med. **54**:694, 1934.

liquefaction and even pyknosis of the entire cell structure. In the cases of acute poisoning there was no glial reaction, but in the chronic cases there resulted extensive astrocytic proliferation with the formation of glial rosettes. Occasional softened areas were also observed. Grayzel limited his experiments to rabbits, in which he produced repeated hypoglycemic convulsions over a period of three months. The resulting damage to the brain tissue varied from areas of hyperchromatic cells to zones of necrobiosis with mild glial proliferation. Grayzel believed that the severity of the cerebral changes depended on the frequency and intensity of the convulsions regardless of the amount of insulin injected. He concluded that even one convulsion, if severe and prolonged enough, was capable of producing definite lesions within the central nervous system. Dünner and his co-workers [10] and Schmid,[11] experimenting with dogs and rabbits, respectively, also reported cerebral changes after a prolonged series of hypoglycemic reactions. Both investigators emphasized the vascular changes. Dünner was impressed by the extensive endothelial proliferation and new vessel formation. Hemorrhages and foci of encephalomalacia were also observed. Schmid likewise found conspicuous vascular changes in his animals. These were associated with prominent marginal gliosis, diffuse swelling of the astrocytes and definite glial reaction within the basal nuclei. During the past few years further observations on the damage to nerve cells in experimental hypoglycemia have been reported by Tani [12] and Weil and his co-workers.[13] Tani produced, besides these cell changes, increase in glia, sclerosis of the cornu ammonis and softening in the substantia nigri. The severity of the changes did not parallel the number of hypoglycemic attacks, since some animals with very few attacks presented severe damage of the brain. Tani suggested that these differences were influenced by the individual variations in the cerebral vascular supply. Weil and his co-workers were particularly impressed by the alterations in the ganglion cells. They described both acute and chronic changes; the former consisting of liquefaction, vacuolation and homogenization; the latter, of shrinkage and pyknosis. There was definite diminution in the number of neurons throughout the cortex. Proliferation of the macroglia was observed in the more chronic experiments. The vascular changes were mild and were associated with some proliferation of the vascular endothelium. The total dose of insulin used in these animals varied from 28 to 402 units. Weil and his associates concluded that

10. Dünner, L.; Ostertag, B., and Thannhauser, S.: Klin. Wchnschr. **12**:1054, 1933.

11. Schmid, H.: Ann. méd.-psychol. **94**:658, 1936.

12. Tani, N.: Zentralbl. f. d. ges. Neurol. u. Psychiat. **80**:30, 1936.

13. Weil, A.; Liebert, E., and Heilbrunn, G.: Arch. Neurol. & Psychiat. **39**: 467, 1938.

the rabbits receiving the larger doses of insulin showed the more extensive histologic changes and that repeated hypoglycemic reactions with small amounts of insulin resulted in little or no damage of the brain, unless the total dose was sufficient to cause such damage.

The motivation of the present studies on hypoglycemia was provided by the widespread chronic alterations observed in the cerebral tissues of the human material examined by me.[14] It was deemed of distinct value to determine whether these cerebral changes could be consistently reproduced experimentally in animals. The chief interest in the present investigations was centered on the chronic and apparently permanent damage of the brain found months after the discontinuation of hypoglycemic shocks. The acute changes recorded were only incidental observations in those animals dying unexpectedly during the experiments.

EXPERIMENTAL INVESTIGATIONS

The 26 rabbits used in these studies were divided into three groups.

The 7 rabbits in the first group were subjected to as severe and prolonged a hypoglycemic reaction as they could safely tolerate. They were then allowed to live as long as one hundred and thirty days and an attempt was made to determine the influence of a single severe reaction on the brain tissue.

The second group contained 13 animals. These were given repeated hypoglycemic reactions at weekly intervals over a period of months, the number of total shocks per animal varying from seven to nineteen. The rabbits were then allowed to live from one to one hundred days, after which a study was made of the possible damage produced in the brain by these repeated cerebral insults.

In the last group were placed 6 rabbits used as controls and kept in the same laboratory as the experimental animals. Hypoglycemia was produced by intravenous injection of insulin into rabbits starved for eighteen hours prior to the onset of each experiment. The individual dose varied from 10 to 30 units. Such a procedure produced very uniform and constant results. From one to two hours after the injection the animals began to manifest the first effects of the hyperinsulinism. Some would become very quiet and unreactive and shortly afterward would pass into a state of complete flaccidity and coma. It was soon learned that the latter condition was very dangerous and required prompt treatment with intravenous injection of dextrose in order to forestall the death of the animal. A second type of reaction consisted of the development of moderate or extreme hyperirritability. External stimuli would at once produce spasticity of the entire animal. After some time these rabbits would begin to manifest convulsive seizures,

14. (*a*) Baker, A. B., and Lufkin, N. H.: Arch. Path. **23**:190, 1937. (*b*) Baker, A. B.: ibid. **26**:765, 1938.

lasting about twenty seconds and recurring every ten to twenty minutes. These reactions were not particularly dangerous but were invariably followed by a comatose condition that was very hazardous. The shock was usually discontinued before the onset of this stage.

HISTOLOGIC OBSERVATIONS

Group 1.—Of these 7 animals only a single rabbit failed to recover from the shock therapy. The rest were killed from one to four months later.

Early Changes: These were limited almost exclusively to the cortical nerve cells, which presented fairly typical acute changes with swelling of the cell body and moderate tigrolysis. Their cytoplasm was usually pale and often vacuolated. The nuclei were, as a rule, intact, although occasionally eccentric in position. More intense damage, when present, resulted in complete fading out of nerve cells to form "ghost cells." When complete destruction occurred, there could be seen large clear spaces containing an occasional cytoplasmic fragment or an isolated nucleus but no demonstrable cell body. These acute and subacute changes were generalized, being at no time limited to any single region. The injured cells were usually interspersed among the unharmed and structurally normal elements. Glial changes were not observed.

Chronic Changes: The damage observed in the brains of those animals which lived for long periods after the single cerebral insult was much less striking than the acute changes. Most of the nerve cells appeared intact. There were a few shrunken, irregular cells within the cerebral cortex. These were markedly pyknotic and contained irregular clumped cytoplasmic granules, tortuous and often fragmented processes and eccentrically placed irregular nuclei.

The macroglia presented mild proliferation. They were moderately increased in number, but their nuclei remained fairly uniform in appearance. This glial proliferation was scattered throughout the brain tissue and often occurred in small localized areas to form tiny glial nodules.

Petechial hemorrhages were not uncommon. These bleedings were discrete, of a ball type and limited primarily to the white substance in the vicinity of the blood vessels. None of these hemorrhages appeared to have caused destruction of the adjacent brain tissue.

COMMENT

The cerebral changes resulting from a single severe prolonged hypoglycemic reaction varied, depending on the stage at which the tissue was studied. Shortly after recovery from the shock there occurred acute swelling of the nerve cells. This early cell reaction appeared to be reversible and tended to disappear in a short time. A few cells, however, were more severely injured and degenerated into a stage of chronic alteration and nonfunction. A reaction on the part of the glial elements was found only as a chronic process but was never conspicuous in these cases of single cerebral insults.

Group 2.—As stated previously, the number of hypoglycemic shocks administered to these animals ranged from seven to nineteen, while the total time that elapsed from the onset of treatment until the brain was removed for study varied from forty-nine to one hundred and ninety days. One hundred and ninety

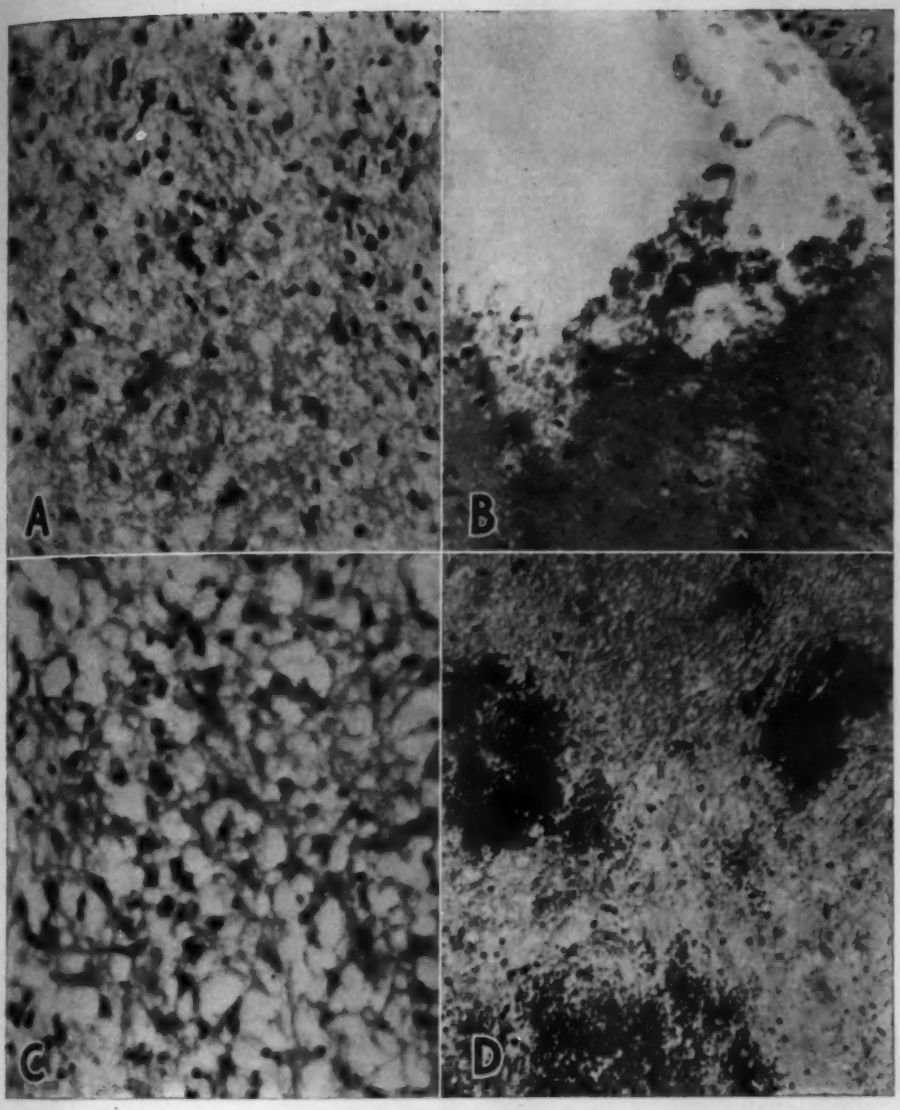
days seemed an adequate period for the appearance of all possible types of permanent cerebral damage.

The alterations in the nerve cells were not conspicuous. As a rule some cells could be found that were shrunken and free from Nissl substance. Their processes were narrowed and irregular, and the entire cell seemed to be collapsed around an irregular, pyknotic nucleus. These changes were scattered with no tendency to localize in any special region of the brain. In a rabbit that had obtained nineteen hypoglycemic reactions the cells in many cortical areas had undergone extensive fragmentation, liquefaction and even actual disappearance. Peculiarly enough, the adjacent cortical cells were intact. The damage of the nerve cells seemed to parallel the number of cerebral insults suffered by the animals, those with the greatest number of attacks of hypoglycemia presenting the most severe chronic alterations of the nerve cells.

The macroglia presented some of the most striking changes. These varied from mild diffuse increase to extensive proliferation, with extreme variations in the size and shape of the young astrocytes (see *A* in figure). Frequently this glial increase remained well localized, producing subpial or perivascular gliosis. The latter was the more common and in some regions obliterated the vessel wall and extended into the adjacent brain tissue. Focal increase in the macroglia unassociated with vessels was also very common. These glial nodules varied in size from some composed of only a few proliferating astrocytes to others containing an extensive cellular reaction covering large areas of tissue. The macroglial reactions appeared to have very little relationship to either the number of hypoglycemic reactions or to the quantity of insulin injected into the animals. It was quite apparent that the macroglial changes were much slower in onset than those of the nerve cells and that sufficient time had to be allowed for them to become apparent.

Injury to the myelin sheaths was also prominent. The milder lesions consisted of focal or diffuse demyelination with accumulation of microglia around and within these softened areas. Many regions had completely lost their tinctorial properties, although actual demyelination was not yet apparent. In the more advanced lesions complete destruction of the tissue had occurred, producing many small irregular cavitations (*B* in figure). These were occasionally lined by glia and often contained red cells that had extravasated from adjacent capillaries. In some areas there occurred very irregular myelin degeneration, giving to the brain tissue a peculiar vacuolated appearance (*C* in figure). In these regions the astrocytes occasionally proliferated to replace the destroyed areas. As in the previous brain changes, the severity and extensiveness of the myelin injury did not seem to be related either to the number of hypoglycemic reactions or to the amount of insulin given the animals.

Vascular changes of some type were observed in almost every case. Vascular congestion was conspicuous. Hemorrhages were an almost constant finding and varied from a few perivascular erythrocytes to extensive bleedings that destroyed brain tissue and occasionally tore through the cortex into the subarachnoid space. The petechiae were almost exclusively of a ball type and usually remained discrete, with little or no injury to the surrounding substance (*D* in figure). Larger hemorrhages were unusual but when present invariably destroyed some of the adjacent brain tissue and produced fairly large areas of surrounding demyelination (*D* in figure). Hemorrhages also occurred within the cortex, where the larger ones often extended through into the subarachnoid space. In some of the bleedings the red cells had already undergone complete hemolysis with the formation of a homogeneous mass. Hemosiderin granules could also be found scattered in some



EXPLANATION OF FIGURE

A, mild diffuse increase of the macroglia; hematoxylin and eosin stain; × 200. The cell nuclei are fairly uniform in size and shape. There is beginning destruction of the myelin tissue.

B, portion of an irregular cavitation observed within the brain of a rabbit subjected to repeated hypoglycemic shock; hematoxylin and eosin stain; × 200. A few erythrocytes are seen in the wall of the cavity.

C, an area of brain tissue demonstrating irregular degeneration of myelin; hematoxylin and eosin stain; × 300. The entire tissue is undergoing degeneration, producing a peculiar vacuolated appearance. Even the cell bodies are involved and cannot be seen clearly.

D, petechiae in the brain of a rabbit subjected to repeated hypoglycemic attacks; hematoxylin and eosin stain; × 300. The hemorrhages are mostly of a ball type and are frequently composed of very dense masses of erythrocytes. Note the beginning demyelination of some of the adjacent brain tissue.

of the injured brain tissue. The quantity of hemorrhages varied from animal to animal. They tended to be most common in those rabbits that had had the greatest number of hypoglycemic shocks.

COMMENT

These histologic studies of the nervous system of rabbits exposed to varying degrees of hypoglycemic shock indicate that such treatment produces widespread severe damage of the brain, which is even more marked in the chronic phases of its development than in the acute stages. In the chronic stages of such injury the nerve cell changes are not conspicuous but are surpassed in intensity by the cerebral petechiae, the areas of demyelination and the diffuse and focal glial proliferation. Chronic cerebral tissue alteration of a similar nature has already been mentioned in sporadic cases in man (Lindsay and others;[4] Hartman;[15] Salm;[3] Morsier and Mozer;[2] Baker[14b]). Any single one or any combination of these alterations is quite capable of producing severe lasting injury of the brain.

The question arises as to the explanation of such extensive damage of the brain. Weil and his co-workers were of the opinion that the total dose of insulin and the severity of the cerebral injury were in some way closely related. Such a correlation was not observed in the present studies. A more likely explanation might be found in the frequency and severity of the possible cerebral anoxemia produced by the hypoglycemic reactions. Impaired nutrition of the brain must accompany hypoglycemia. It is well known that cerebral changes follow circulatory disturbances due to impairment of cerebral circulation. Such alterations have been described in man by Döring[16] and Kammy.[17] In persons dying from obstruction of the cerebral circulation they observed injury of nerve cells, many small diffuse areas of demyelination and focal areas of glial proliferation. Similar changes were produced experimentally in cats by Gildea and Cobb[18] by ligation of the carotid arteries. In hypoglycemia it is probable that a similar circulatory disturbance results, only here it is of a qualitative nature inasmuch as the blood reaching the cerebral tissues is deficient in the proper nutritive materials.

SUMMARY

Rabbits were subjected to repeated hypoglycemic reactions and their nerve tissues studied months after the last reaction in order to evaluate the resulting chronic and permanent damage of the brain.

15. Hartman, F. W.: J. A. M. A. **109**:2116, 1937.
16. Döring, G.: Virchows Arch. f. path. Anat. **296**:666, 1936.
17. Kammy, E.: Beitr. z. path. Anat. u. z. allg. Path. **100**:248, 1938.
18. Gildea, E. F., and Cobb, S.: Arch. Neurol. & Psychiat. **23**:876, 1930.

Although nerve cell changes did occur, these were by no means as striking as the cerebral hemorrhages, the areas of demyelination and encephalomalacia and the glial reactions.

No correlation could be found between the total dose of insulin administered and the severity of the damage of the brain.

It is suggested that the cerebral damage in hypoglycemia might be due to a qualitative circulatory disturbance inasmuch as the blood reaching the brain is deficient in the proper nutritive materials.

Federal Aid students assisted in the care of the animals during these experiments.

NEW FORMATION OF ELASTIC TISSUE IN ADHESIONS BETWEEN SEROUS MEMBRANES AND IN MYOCARDIAL SCARS

C. H. BUNTING, M.D.
MADISON, WIS.

Some years ago, in collaboration with Dr. Gaylord Coon, I made some preliminary experiments in an attempt to find under what conditions elastic tissue might be laid down in repair tissue. These experiments went only so far as the demonstration that in the scar tissue that resulted from the healing of ulcers of the skin of rabbits there was no new formation of elastic tissue. There was, however, slight evidence of growth of the cut fibers at the edges of the wounds in the form of a brushlike extension of short delicate fibrils from these fibers. The only other positive observation in the series of sections was that the youngest connective tissue fibers, i. e., the ones immediately around the fibroblasts in the developing repair tissue, were argentaffin in specimens stained with the Bielschowsky silver stain, while the more distant, older fibers took the orange-yellow stain of collagenous tissue.

At that time the idea was present in our minds, from the distribution of elastic tissue in the body and from its new formation in such locations as the intima of an artery, the seat of endarteritis, that if one could subject developing connective tissue to protracted alternations of tension and relaxation, the development of elastic fibers might be obtained. However, an appropriate experimental setup did not present itself at that time, and the study was dropped.

Recently it occurred to me that in a variety of pathologic processes in man nature has performed the desired experiment, and this has led to the present study. The experiment par excellence is that in which vascularized fibrous adhesions have resulted from the organization of an adhesive fibrinous exudate in a serous cavity. Such connective tissue strands, joining the two layers of the pericardium, are subject to variation in tension with every heart beat; and those of the pleura, with every respiration. Myocardial scar tissue, likewise, is subjected to variations in tension during the cardiac cycle.

The first case studied was that of a woman of 46 who gave a history of rheumatic fever at the age of 10 years and "heart trouble" from

From the Department of Pathology of the University of Wisconsin.

that time on. At postmortem examination there were found, in addition to valvular lesions and a hypertrophied heart (480 Gm.), a scarred myocardium and chronic fibrous adhesions between the parietal and the visceral layer of the pericardium over the left ventricle and left auricle. Histologic sections of these adhesions stained by the Weigert elastic tissue stain show a well marked development of elastic tissue (fig. 1). There is a considerable network about the new blood vessels and adjacent to them, but also heavy strands, independent of the blood vessels, are found extending in a line parallel to the line of tension in the adhesion. While much of this has the sharp double contour of active, healthy elastic fibers, in many places there is evidence of degeneration. The fibers are broken and granular and present a fuzzy appearance. The visceral pericardium itself shows a marked increase in elastic tissue beneath the adhesions. There are heavy fibers not only in the submesothelial fibrillar tissue but also in the deeper layers of the membrane and even among the adipose tissue cells.

Sections of a myocardial scar in the same heart showed, with the Weigert elastic tissue stain, an extremely dense development of heavy elastic fibers running parallel to one another and to the intervening collagenous fibers and adjacent muscle fibers. The fibers were so numerous and so closely packed that the specificity of the stain was under suspicion until close observation showed at the edges the characteristic doubly contoured branching fibers (fig. 2 *B*).

These specimens are not unique. Figure 2 *A* is from an elastic tissue stain of the scar of an old infarct in the 1,060 Gm. heart of a 54 year old man with generalized arteriosclerosis, including marked involvement of the coronary arteries. The section is cut somewhat obliquely to the direction of muscle and scar tissue but indicates perhaps better the typical morphologic picture of the cut heavy elastic fibers than a directly parallel section.

In all other cases of adherent pericardium and of myocardial scars examined, new formation of elastic fibers has been found. Such new growth is present not only in fibrillar adhesions between the serous surfaces but also in diffuse or universal adhesions. In all cases the direction of the main elastic fibers has been parallel to the apparent line of tension, i. e., parallel to the connective tissue bundles or to the heart muscle fibers.

In the fibrous thickening of the endocardium of the walls of dilated chambers of the heart there is also an increased elastic fiber content, but in this location it is more difficult to determine that it is not the result of a development from the preexisting normal elastica of the endocardium.

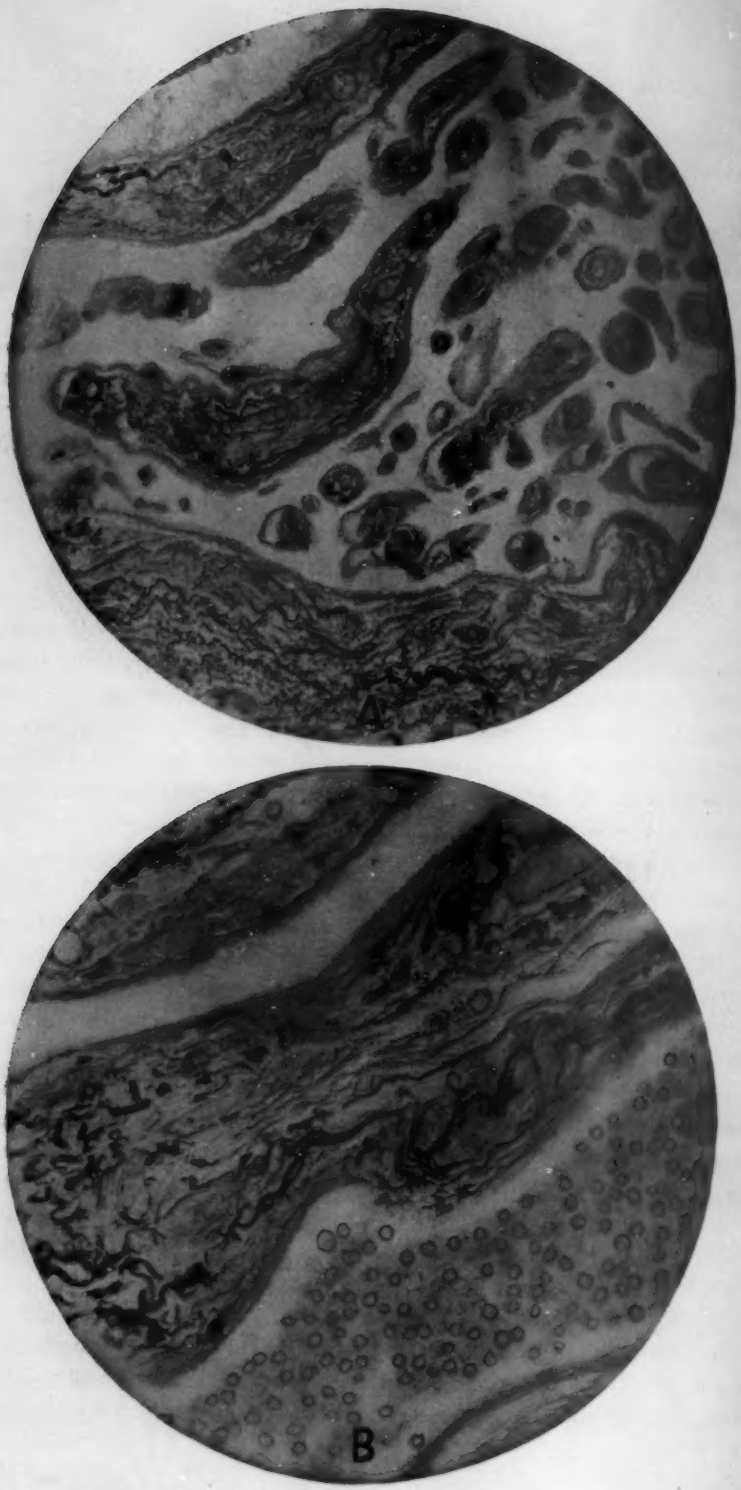


Fig. 1.—*A*, low power photomicrograph of visceral pericardium and fibrous adhesions; *B*, high power photomicrograph of a single adhesion in the same case; Weigert elastic tissue stain.

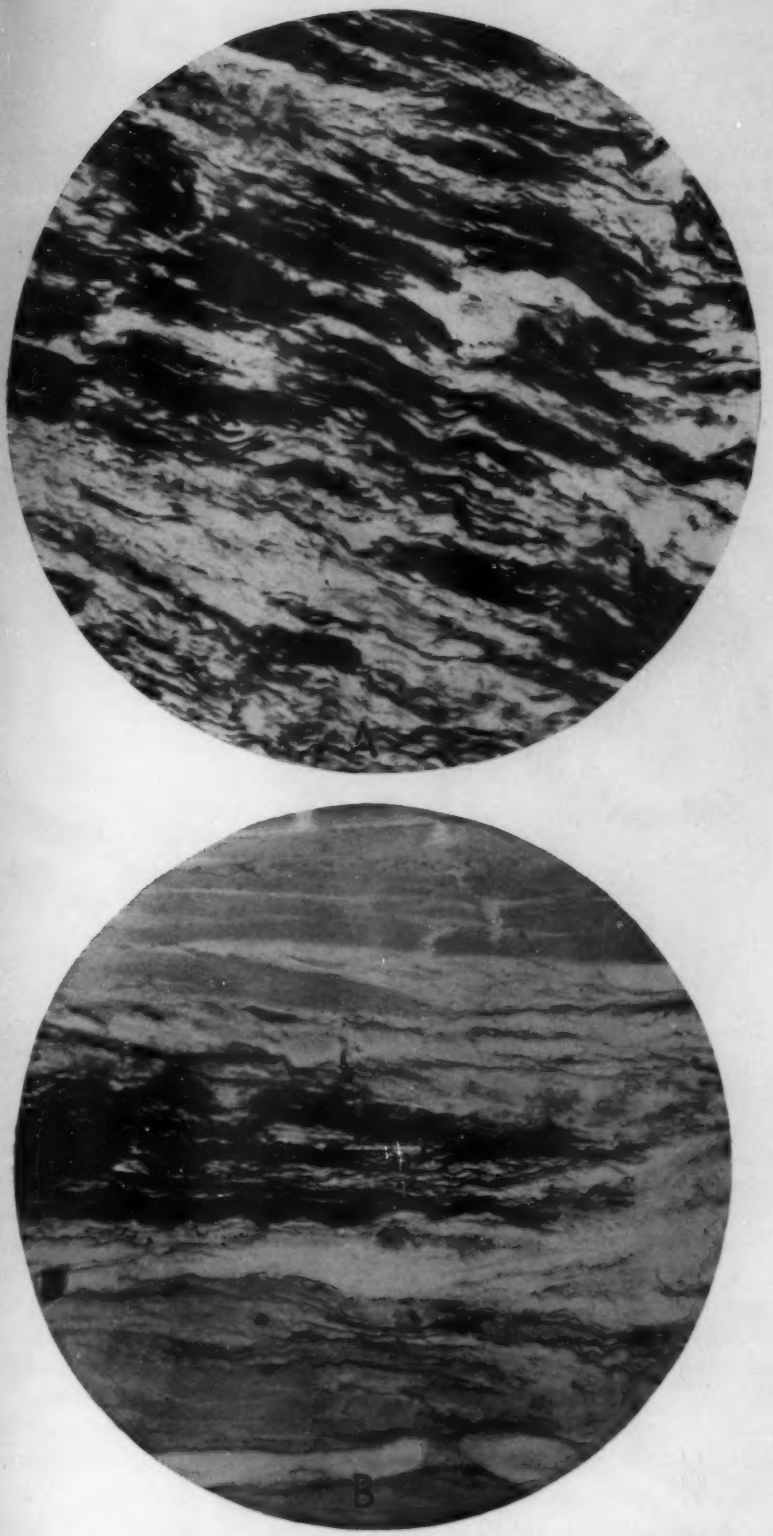


Fig. 2.—*A*, photomicrograph of old scar of a cardiac infarct; Weigert elastic tissue stain; *B*, edge of myocardial scar in the same case as tissue shown in figure 1.

A similar development of elastic tissue, although somewhat less extensive, has been found in all pleural adhesions which have been examined, both the diffuse and the fibrillar type. The new fibers are found not only in connection with the new capillaries, among the older of which they form a definite layer of the vessel wall, but among the collagenous fibers and to a large extent definitely parallel to them. From previous

Fig. 3.—High power photomicrograph of chronic fibrous pleural adhesions; Weigert elastic tissue stain.

experimental work this appears to indicate that they are laid down parallel to the line of tension.[1] In figure 3 is reproduced a typical picture of pleural fibrillar adhesions stained by the elastic tissue stain. At the left of the photograph it may be seen that the elastic fiber of the adhesion is continuous with a newly developed elastic fiber which lies outside of the elastica propria of the visceral pleura and is unconnected with it.

1. Bunting, C. H., and Eades, C. C.: J. Exper. Med. **44**:147, 1926.

A feature of certain pleural adhesions which deserves further study is a new growth of smooth muscle both in the visceral pleura and in the adhesion itself. In the pleura the muscle cells appear to be associated chiefly with the lymphatics. In the adhesions some bands of muscle are in association with newly formed vessels and others are quite independent of them.

In a fibrillar adhesion between the spleen and diaphragm a definite but not extensive new formation of elastic tissue was found.

Checking these findings with old repair tissue in man, one finds that where the latter is not subject to any marked intermittent strain the findings agree with those in the rabbit. Elastic tissue is not laid down among the collagenous bundles. There may be some new tissue of elastic type laid down in association with the new vessels, parallel to them, and usually perpendicular to the new connective tissue.

Whether or not these findings are sufficient to establish the thesis that tension, and probably intermittent tension of the tissue, is the factor that determines the formation of elastic tissue in repair may be questioned. The development of elastic tissue in the lung, however, seems to follow the establishment of respiratory activity if one may accept as fact that there are intrauterine respiratory movements. In the lungs of a 6.5 cm. fetus there was well developed elastic tissue only in the vessels, with a few delicate fibrils beginning to show in the walls of the trachea and larger bronchi. In 2 fetuses 30 cm. in length there were a delicate submesothelial line of elastic fiber in the pleura, a subepithelial layer in the larger and medium-sized bronchi and few dots and short delicate fibrils (best brought out by an orcein stain) in the respiratory bronchioles and ductuli alveolares. In 2 fetuses of 41 and 42 cm. length, the pleural elastic layer consisted in the main of a single layer, but in some places it was doubled. Within the lung the musculoelastic layer of the respiratory bronchioles was sharply developed; their alveoli, however, showed no elastic tissue in the walls.

This condition persists until the time of birth, and, in fact, until some months after birth—for it is only in children 7 months old that I have found the beginning of elastic tissue in the alveolar walls, and only in children 10 months old that it is well developed. In this same interval the elastica propria of the pleura has become a double or even triple layer structure with interlacing fibrils. It seems, then, that elastic tissue in the lung is developed only with increasing activity of that organ.

It was not the purpose of this study to try to answer the old question as to whether elastic tissue is laid down as such by connective tissue cells or whether preexisting collagenous fibers are converted into elastic tissue. I doubt that the specimens studied can give a satisfactory answer. One may convince oneself from the relation of the elastic fibers to

collagenous bundles in an old myocardial scar that the elastic fibers must be due to a conversion of the collagenous tissue and then be confronted with a more recent pleural adhesion in which terminal edema had widely separated the elements and in which the new elastic fibers are so closely applied to the young fibroblasts that it seemed that they must be a direct result of cellular activity. It is, of course, possible that elastic fiber is formed by both methods.

In conclusion I would note that in adhesions between serous membranes and in scars, where both are subject to alternations of tension and relaxation abundant elastic tissue is developed with its main direction parallel to the line of tension. The excess development in such areas in contrast to that in scar tissue in other locations seems to indicate the importance of this mechanical stimulus to its development. Other factors are of course not excluded.

HISTOLOGIC STUDY OF REPARATION OF EXPERIMENTALLY PRODUCED DEFECTS IN CALVARIUMS OF RATS

CHARLES J. SUTRO, M.D.*
AND
SHELDON A. JACOBSON, M.D.
NEW YORK

That fractures and defects of the calvarium heal slowly or not at all has been a frequent observation. Most of the reports in the literature are based on clinical investigations in man.[1] The lack of exact knowledge concerning the processes involved seemed to warrant an experimental investigation into the matter.

The study was conducted in the following fashion:

Two groups of albino rats, unselected for sex and averaging, respectively, around 80 and 150 Gm. in weight, were subjected to operation. With the rat under amytal anesthesia, a midline sagittal incision was made in the skin over the calvarium. The point of the scissors was pressed through the parietal bone on one side, with as little damage to the brain as might be, and a triangular piece of bone measuring approximately 3 mm. on each side was removed. The skin was then sutured. The animals were kept on stock diets and killed at the periods of time after operation shown in the table.

For the histologic examination, the head was removed, sawed partly in half along the midline and fixed in Zenker's solution, and then both sides were sectioned and the sections stained with hematoxylin and eosin.

OBSERVATIONS

Young Rats (80 Gm.).—It was found that a few fragments of bone were left as a result of the operative procedure. There was a considerable amount of hemorrhage but only superficial destruction of the

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*Frauenthal Orthopedic Travel Fellow (1937).
From the Laboratory Division, Hospital for Joint Diseases.

1. Meade, R. H.: Med.-Chir. Tr. **23**:390, 1840. Watson, A.: Edinburgh M. J. **63**:302, 1845. Weist, J. K.: Cincinnati Lancet & Obs. **9**:155, 1866. Shoemaker, G. E.: Philadelphia M. Times **13**:745, 1883. Billroth, T.: General Surgical Pathology and Therapeutics, translated and revised by C. E. Hackley, New York, D. Appleton and Company, 1886. Helferich, H.: On Fractures and Dislocations, New York, William Wood & Company, 1899. Koenig, F.: Lehrbuch der speciellen Chirurgie, Berlin, A. Hirschwald, 1875, vol. 1, p. 38. Macewen, W.: The Growth of Bone, Glasgow, J. Maclehose & Sons, 1912. Naffziger, H. C., and Glaser, M. A.: Surg., Gynec. & Obst. **51**:17, 1930. Wakeley, C. P. G.: Practitioner **127**:75, 1931. Ireland, J.: Arch. Surg. **24**:23, 1932. Troitzky, W.: Ztschr. f. Morphol. u. Anthropol. **30**:504, 1932. Wright, L. T.; Greene, J. J., and Smith, D. H.: Arch. Surg. **27**:878, 1933. Echlin, F.: ibid. **28**:357, 1934. Earley, D. E.: J. A. M. A. **104**:2332, 1935.

brain. None of the animals exhibited obvious neurologic defects. The hemorrhage was quickly resorbed. After a lapse of eight days the gap was closed by a membrane of usually not very cellular fibrous connective tissue. There was localized necrosis of the fractured ends. The pericranial membrane had undergone proliferation to a moderate degree, as had the dura. Both lateral sides of the skull showed this change. At this early period ossification had already commenced in the periosteum near the fractured edge but was exceedingly limited in extent. The pericranium was the more active in this phenomenon. By the thirteenth day the corners of the defect had been completely bridged by new bone, while the wider part had granulation tissue only (fig. 1 *A*).

In the eighth week newly formed fiber bone had reduced the cavity by half. Near the defect there was little periosteal activity on either the

Interval Between Experimental Production of Defect in Rat's Skull and Histologic Examination of Repair of Defect

Young Rats (80 Gm.)		Adult Rats (150 Gm.)	
Rats	Interval	Rats	Interval
1	4 days	1	4 hours
1	8 days	1	2 days
1	13 days	1	4 days
1	53 days	3	8 days
1	80 days	1	11 days
1	90 days	2	14 days
1	140 days	1	17 days
1	150 days	1	21 days
1	180 days	1	30 days
2	300 days	1	150 days
		1	175 days
		1	210 days
		1	300 days

outside or the inside of the calvarium. After five months the site of operation was no longer identifiable.

Adult Rats (150 Gm.).—The initial appearance of the lesion was similar to that already described. By the fourth day there had been some pericranial proliferation on both lateral sides of the skull. On the eighth day also the two pictures were very similar (fig. 1 *B*). In different rats the dura or the pericranium might assume the main burden of bony replacement. One case, indeed, was noted in which there was already considerable new bone in the defect.

In the ensuing weeks the picture changed slowly (fig. 2 *A*). Shelves of newly formed bone appeared on the fracture edges and began to bridge the gap. This was usually a markedly asymmetric process, one side participating hardly at all (fig. 2 *B*). Canalization of the new bone had begun.

In 2 rats examined fourteen and seventeen days after operation, respectively, buttons of excised bone had inadvertently been left in place. These were serving as scaffolds for osteogenesis—in one rat, by the periosteum of the fragment, and in the other, by invasion of the diploe from the broken ends (fig. 3). A thirty day period brought about no advance of the process.

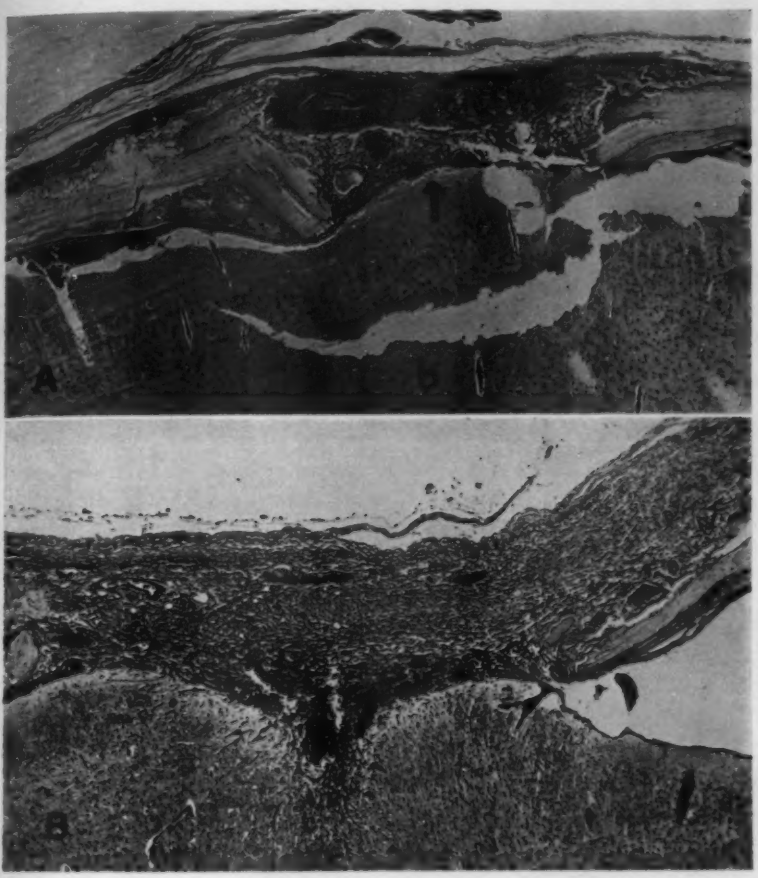

Fig. 1.—*A*, photomicrograph showing the narrower part of the defect bridged by new bone; × 30. Note (arrow) the newly formed dural membrane. Brain is seen in the lower half of the picture (*b*). *B*, photomicrograph showing a densely cellular mass of fibrous connective tissue filling in the gap after an interval of eight days; × 20.

Some of the histologic preparations of animals killed at one hundred and seventy-five days showed the defect still present and bridged only by a rather poorly cellular connective tissue membrane. The pericranium

Fig. 2.—*A*, photomicrograph showing absence of osteogenesis in the calvarial gap after fourteen days; × 30. Note the free piece of necrotic original calvarial bone. *B*, photomicrograph revealing a tongue of fiber bone spreading across the defect (arrow) after an interval of eleven days; × 20.

Fig. 3.—This photomicrograph reveals new bone formation about the depressive fragments of calvarium; × 30. Note again the realinement of the newly formed dural membrane (arrow).

and dura were quiescent and were being restored in the defect. There had been thickening of the fractured edges with healing in some necrotic fragments. Other slides, probably from narrower portions of the incisions, showed complete bony continuity, with excessive bone on the inner side.

It is noteworthy that nowhere was true callus encountered. There were indeed a very few feeble attempts at metaplastic ossification on the part of the connective tissue which closed the wound in the calvarium. Nowhere, however, did this reach an extent or vitality comparable to that commonly seen after fracture of a long bone. Indeed, it was for the most part so sluggish in appearance, so poorly cellular and ill vascularized that it hardly merits the appellation of granulation tissue. It is perhaps worth mentioning that cartilage did not develop. On the contrary, where bony bridges appeared, they seemed to be the result of a steady progression, probably through the intermediation of osteoblasts from the broken edges, outward across the opening. Infection occurred in a few instances and seemed to exercise a depressing influence on the healing process. Only once was it severe. The finding of bony thickening of the fracture edges may be correlated with the occasional roentgenographic observation of increased density along the old fracture lines in man.[2]

SUMMARY

The reparation of experimentally produced defects in the calvariums of rats was slow. In the young rats the defects were reduced by newly formed fiber bone to only one half of the original area after a period of eight weeks. In the older rats the defects were noted even after one hundred and seventy-five days.

2. Stewart, W. H.: Brit. J. Radiol. **30**:399, 1925. Illig, W.: Fortschr. a. d. Geb. d. Röntgenstrahlen **43**:76, 1931. Lindemann, E.: Arch. f. Ohren-, Nasen- u. Kehlkopfh. **135**:25, 1933. Glaser, M. A., and Blaine, E. S.: J. A. M. A. **107**:21, 1936. Vance, R. G.: Am. J. Roentgenol. **36**:744, 1936.

CHEMOTROPISM OF HUMAN EOSINOPHILIC POLYMORPHONUCLEAR LEUKOCYTES

EDGAR S. INGRAHAM, M.D.
AND
WILLIAM B. WARTMAN, M.D.
CLEVELAND

Although a great deal is known about the function of neutrophilic leukocytes in inflammation, this is far from being true in regard to polymorphonuclear eosinophils. As far as can be determined by search of the literature it is not known whether these cells in man show chemotaxis either to bacteria or to those animal parasites which cause eosinophilia. Rosegger[1] reported that Ascaris attracts eosinophils obtained from horse blood. His report is based on a cinematographic study of the behavior of a very few cells, and the results cannot be analyzed statistically. The lack of knowledge concerning eosinophils is, of course, due to the difficulty of obtaining enough cells for adequate experiments. Recently we had the good fortune to see 2 patients who supplied us with great numbers of polymorphonuclear eosinophils. One of them had eosinophilic myelogenous leukemia and the other dermatitis herpetiformis. The chemotaxis of eosinophils obtained from these patients was studied in vitro with bacteria, Trichinella spiralis and Witte's peptone as sources of attraction. The chemotropism shown was compared with that of polymorphonuclear neutrophils obtained from healthy persons.

METHODS

The eosinophils used in the experiments were obtained from two sources: (1) the fluid of fresh bullae of the patient with dermatitis herpetiformis; (2) blood obtained by puncture of a finger of the patient with myelogenous leukemia of eosinophilic type. Blood obtained in the same manner from healthy adults was used as a source of neutrophils. The fluid of the bullae contained 76 per cent eosinophils, many of which were degenerate and moved poorly. The patient with leukemia had a total leukocyte count of approximately 30,000, with 70 to 80 per cent adult eosinophils, which morphologically were identical with the eosinophils seen in the circulating blood of healthy adults. The attracting substances used were: (1) twenty-four hour cultures of Staphylococcus aureus; (2) dried, ground Trichinella spiralis; (3) Witte's peptone. The peptone was adsorbed to titanium dioxide in order to have a visible target.

From the Institute of Pathology, Western Reserve University and University Hospitals.

1. Rosegger, H.: Ztschr. f. d. ges. exper. Med. **85**:712, 1932.

A fragment of one of the attracting substances was fixed on a glass slide by drying. A drop of blood or of fluid from a bulla was placed on a cover slip and spread over the test substance, which thus formed a small target on the slide. The movements of leukocytes near the target were observed under the microscope, and the course of each cell was recorded with the aid of a drawing ocular at one minute intervals for a period of ten minutes (fig. 1). All the observations were made in a warm chamber at 37 C.

In these experiments the measure of chemotropism was defined as the net approach in microns per minute of each cell toward the test substance. In each group of experiments the results were expressed as the mean value of chemotropism of all the cells observed. The random movement of cells in fields remote from the target was observed in each preparation in order to make certain that directional currents were not present (fig. 2). In the case of Witte's peptone, control experiments were made using plain titanium dioxide.[2] In order to set up a standard of comparison, polymorphonuclear neutrophils obtained from the finger blood of healthy adults were tested with the same substances as the eosinophils.

RESULTS

The first series of experiments was designed to test whether eosinophils are attracted by two common bacteria, namely, Staph. aureus and Escherichia coli. The results are shown in table 1. The mean value of chemotropism for 35 eosinophils from the blood of the patient with leukemia, tested against Staph. aureus, was + 9.0, with a standard error of ± 1.51, whereas the value of chemotropism for 67 neutrophils from healthy adults was + 7.5, with a standard error of ± 1.20. The difference of the means with its standard error was 1.5 ± 1.94. Since the difference of the means is not more than twice its standard error these results are not significantly different from each other. In other words, eosinophils from the patient with leukemia were attracted as strongly as neutrophils from presumably healthy persons.

The eosinophils obtained from the fluid of the bullae were attracted less strongly by Staph. aureus than were normal neutrophils. Although this difference is statistically significant, it is probably of little real significance because of the fact that these eosinophils were not healthy cells but showed obvious evidences of degeneration and poor locomotion. In the second column of table 1 it is seen that eosinophils from the bullae were attracted by Esch. coli.

In the second series of experiments the effects of two substances known to produce eosinophilia in experimental animals were tested. The results are shown in table 2. Fifty-four neutrophils from healthy adults, tested with dried T. spiralis, gave a mean value of chemotropism of + 7.0 ± 0.33, and 224 eosinophils from the blood of the patient with leukemia

2. Titanium dioxide has previously been shown to be chemotactically inert (unpublished data).

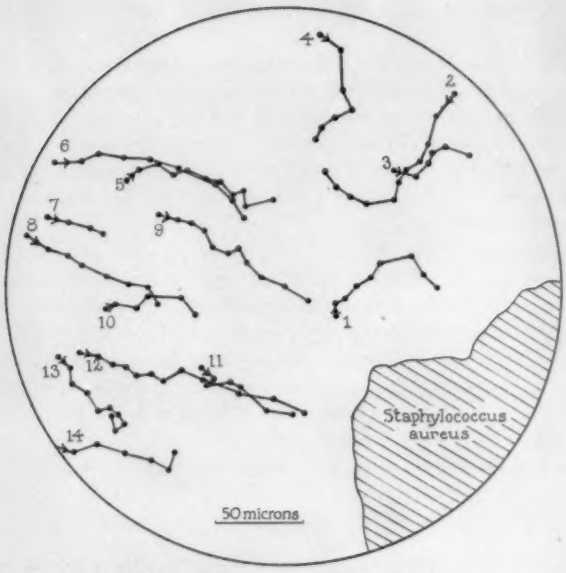

Fig. 1.—A camera lucida record of the attraction of human eosinophilic polymorphonuclear leukocytes to Staphylococcus aureus. The cells were obtained from the blood of a patient with myelogenous leukemia of eosinophilic type. The numbers represent the positions of the eosinophils when first observed, and the dots show successive positions at one minute intervals. The cells are moving toward the bacteria.

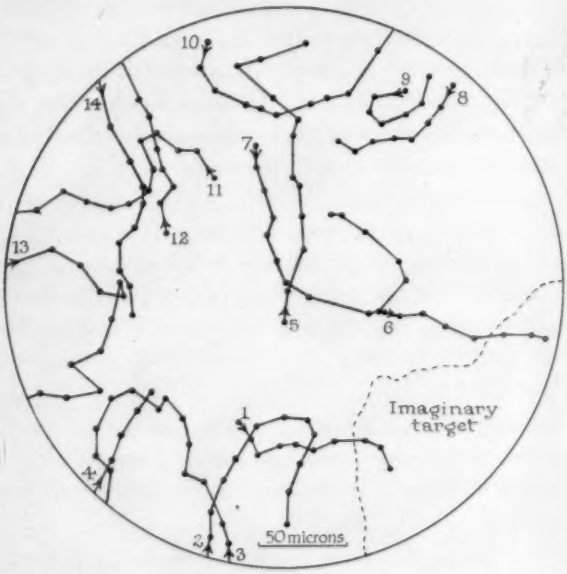

Fig. 2.—A camera lucida record showing random motion of eosinophils. This is the control of the experiment shown in figure 1. The imaginary target is obtained by projecting the outline of the bacterial target on the microscopic field. The cells show no directional movement.

gave a mean value of $+ 5.8 \pm 0.99$. The difference of the means and the standard error is 1.2 ± 1.06. Consequently there was no significant difference in the chemotactic behavior of the two types of cells. Similar results were obtained when Witte's peptone on titanium dioxide was employed as the test substance.

COMMENT

These experiments indicate that in vitro eosinophilic polymorphonuclear leukocytes show the same degree of positive chemotropism as do

TABLE 1.—*Chemotropic Response of Polymorphonuclear Eosinophils to Bacteria*

	Staphylococcus Aureus		Escherichia Coli	
Polymorphonuclear Leukocytes Used	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error
Neutrophils from blood of healthy adults	67	$+7.5 \pm 1.20$	52	$+5.8 \pm 0.50$
Eosinophils from blood of patient with leukemia	35	$+9.0 \pm 1.51$		
Eosinophils from bulla fluid of patient with dermatitis herpetiformis	92	$+4.6 \pm 0.86$	63	$+4.7 \pm 1.20$

TABLE 2.—*Chemotropic Response of Polymorphonuclear Eosinophils to Trichinella Spiralis and Witte's Peptone*

	Dried, Ground Trichinella Spiralis		Witte's Peptone on Titanium Dioxide	
Polymorphonuclear Leukocytes Used	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error
Neutrophils from blood of healthy man	54	$+7.0 \pm 0.33$	16	$+9.8 \pm 1.04$
Eosinophils from blood of patient with leukemia	224	$+5.8 \pm 0.99$	75	$+8.6 \pm 0.02$

neutrophils. It is also interesting to observe that eosinophils are not attracted more strongly by T. spiralis than by bacteria; in fact they are attracted less strongly by this parasite than by Witte's peptone. Although the leukocytes employed are not strictly comparable to the eosinophils seen in normal human blood or in an inflamed area, nevertheless they are probably similar cells. It is well known that polymorphonuclear eosinophils appear in both acute and chronic inflammations, but the manner in which they get to the inflamed tissues is not clear. Some believe that the eosinophils originate within the tissues either from previously existing cells or as derivatives of cells which have infiltrated from the blood. However, since it has been shown that eosinophilic

polymorphonuclear leukocytes possess positive chemotropism, it seems at least possible that they may be actively attracted to a site of inflammation in the same fashion as the polymorphonuclear neutrophils.

Eosinophilic polymorphonuclear leukocytes are also capable of phagocytosis. This has been shown by several workers,[3] and we have been able to confirm their observations. Thus it is seen that the eosinophil behaves much like the neutrophil in that it shows both phagocytosis and chemotropism. In contrast to these two cells is the lymphocyte which, so far as is known, shows neither phagocytosis[3a] nor chemotropism.[4]

SUMMARY

The chemotropism of human polymorphonuclear eosinophils was studied in vitro. The cells were obtained from a patient with eosinophilic myelogenous leukemia and from one with dermatitis herpetiformis. The chemotactic reaction of eosinophils to bacteria was found to be as strong as that of normal neutrophilic leukocytes. Eosinophils were attracted less strongly by material from an animal parasite, T. spiralis, than by Witte's peptone. The origin of eosinophils in the tissues is discussed.

Dr. Joseph T. Wearn gave us permission to study the 2 patients mentioned in this article.

3. (*a*) Strumia, M. A., and Boerner, F.: Am. J. Path. **13**:335, 1937. (*b*) McDonald, S., and Shaw, A. F. B.: Brit. M. J. **2**:966, 1922.

4. Dixon, H. M., and McCutcheon, M.: Arch. Path. **19**:679, 1935.

GENESIS OF INFARCTION

LOUIS LOEFFLER, M.D.
DECATUR, ILL.

The genesis of infarction in the human body is not as yet clearly understood. The question still remains why in one organ—the lungs or intestines, for example—the infarct is practically a hemorrhagic one, while in other organs—the kidneys or spleen—an anemic infarct is quite as regularly found. It is difficult to explain why stoppage of the blood supply to one organ may produce hemorrhagic necrosis, while stoppage of the supply to another produces anemic necrosis in spite of collaterals present.

Previous experiments reported in this journal [1] showed that ligature of the mesenteric artery in rats produces anemic necrosis, while ligature of the portal veins produces a lesion resembling hemorrhagic infarction.

Because the animals lived only for hours after the ligature of those large vessels, it was felt necessary to continue the experiments.

The following experiments on intestinal infarction may be reported because of their simplicity and unequivocal results and because the tissue changes are much more distinct than those in previous experiments. The results seem to be well comparable to findings in the human body.

PROCEDURES

Altogether 60 animals, including 12 guinea pigs and a few rabbits, but mostly white rats, have been used.

Sodium amytal and ether were the anesthetics used; the latter was found to be more convenient.

First Series (10 Rats and 6 Guinea Pigs).—A loop of small intestine about 4 to 6 cm. in length, together with its mesentery, was firmly tied with a thick thread. The tying was done as quickly as possible, within a fraction of a second. After a few minutes the loop contracted both in length and in width. The little blood of the loop drained into the mesenteric veins up to the tying point. Only the smallest intramural or serosal veins of the loop remained visible; these were, however, greatly narrowed.

After three to six hours the loop began to look dull, gray and flabby. There was no change in the lumen.

The next day the loop was as white as pale pus, entirely without tonus and dull in appearance; this was even more pronounced forty-eight to seventy-two hours after the operation. The lumen was narrow and empty (fig. 1 *A*).

From the Pathological Laboratory of the Decatur and Macon County Hospital.

1. Loeffler, L.: Arch. Path. **22**:755, 1936.

All animals died after one to three days from intestinal obstruction. The loops above the obstruction were found dilated and reddened. There was a thin purulent fibrinous membrane around the tied loops and adjacent tissue, mostly omentum; however, there was no general peritonitis.

Microscopically, the signs of necrosis were obvious after twenty-four hours, beginning with loss of structure and falling apart of cells (fig. 2 *A*). This was

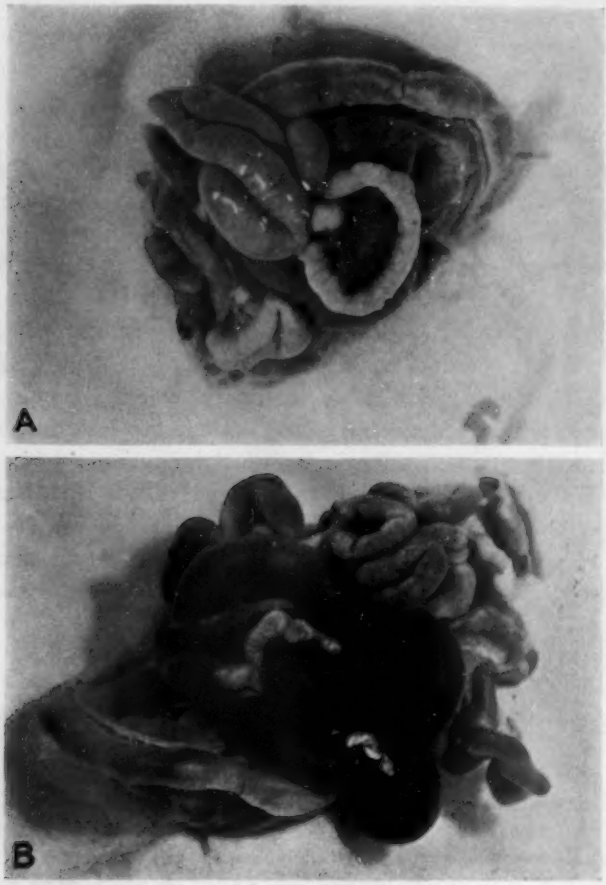

Fig. 1.—*A*, anemic necrosis of an intestinal loop of a guinea pig one day after ligation. The ligature was suddenly and completely tied. About actual size. *B*, hemorrhagic infarction of an intestinal loop in a guinea pig one day after ligation. The ligature was slowly and incompletely tied. About actual size.

followed by loss of nuclear staining of the mucosa, the musculature retaining its staining power until death. Here and there small serosal veins could be found filled with well preserved erythrocytes. The serosa was smooth or covered with fibrin and leukocytes (fig. 2 *B*).

Second Series (10 Rats and 6 Guinea Pigs).—An artificial volvulus was produced. An intestinal loop was held between two fingers, rolled around itself in a spiral fashion and loosely fixed in that position by a string at the base and a suture

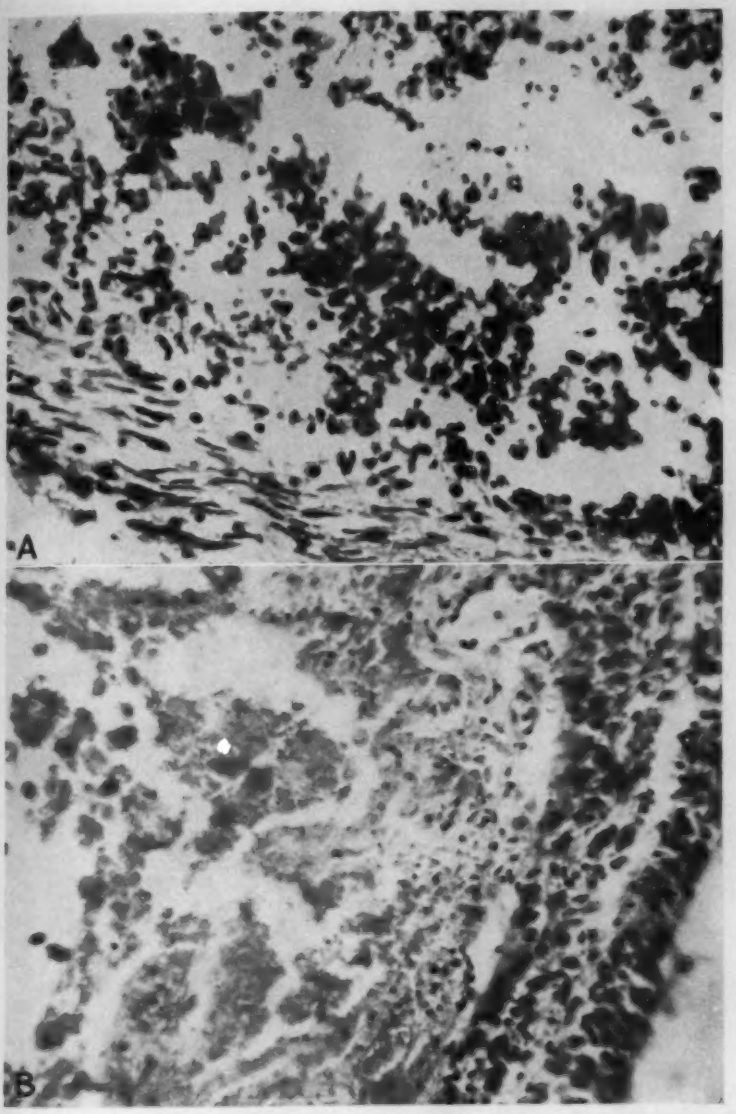

Fig. 2.—*A*, beginning anemic necrosis one day after ligation: *B*, complete necrosis three days after ligation; × 200. A layer of leukocytes and fibrin is seen at the right in *B*.

through the serosa. In the guinea pigs it was only necessary to set two ligatures with a thick thread, the first near the base of the mesentery and the second near the intestinal wall about 1 to 2 cm. distant from the first.

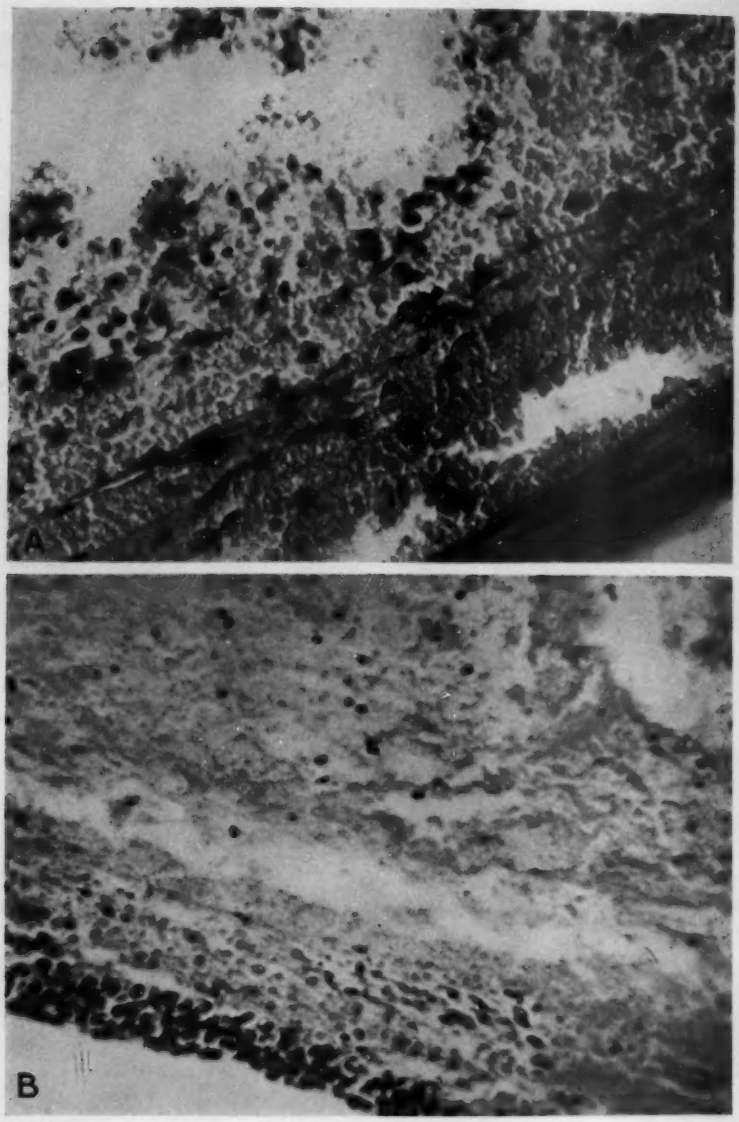

Fig. 3.—*A*, hemorrhagic infarction of an intestinal loop one day after ligation; × 200. Masses of erythrocytes are seen within the broken-up wall. *B*, hemorrhagic infarction three days after ligation; × 200. Necrosis is completed. The shape of the erythrocytes is at places still seen. A layer of leukocytes at the bottom of the picture is well stained in sharp contrast to the necrosed wall.

It was necessary to observe the circulation in the mesentery and see that all ligatures were only narrowing and not entirely obstructive of the blood supply. Pulsation in the dilated vessels was noticed where the mesentery bordered the intestinal wall.

Extreme care, however, was not necessary; there was no failure in any of the experiments—quite an unusual feature in experimental work. Each experiment was finished in from fifteen to twenty minutes.

After twenty-four hours the intestinal loop on top of the volvulus, most often both loops, were greatly dilated, dark black-red, hemorrhagic. The loops were filled with turbid bloody exudate. The serosa was dull (fig. 1 *B*).

In some cases there was local peritonitis around the loop with fibrinous adhesions after two or three days but no general peritonitis.

All animals died after three days from intestinal obstruction, as did those of the first series.

Microscopically, the typical picture of hemorrhagic infarction was seen. All vessels were greatly dilated and filled with erythrocytes, and there was complete destruction of the mucosa and submucosa by masses of extravasated erythrocytes (fig. 3 *A*). Nuclei ceased to stain after two or three days; pyknotic and broken-down nuclei were found in abundance. The musculature was less hemorrhagic than the inner layers and retained its staining power until death. The intestinal wall was thinned (fig. 3 *B*).

COMMENT

In the first series of experiments an intestinal loop was shut off suddenly and completely from its blood supply. The result was without exception anemic necrosis of the intestinal loop. This is what one would expect from such an operation; an organ deprived of its blood must be anemic and must undergo necrosis in due time. It is understood that a short time after the ligature has been set blood is still in the vessels giving the tissue a slightly red color. The red changes into white with advancing destruction and dissolution of tissue and erythrocytes. After one to two days not a trace of red remains in any one of the loops.

The observed contraction of the muscular tissue of the wall is secondary to the anemia. Contraction sets in after a few minutes, driving the remaining blood into the mesenteric veins. The muscular contraction, however, cannot be held entirely responsible for the anemia observed. It is the anoxemia which produces the contraction. The musculature finally loses its tonus; the wall becomes flabby. The mucosa disintegrates first. Then the cells of the mucosal glands fall apart and gradually lose their nuclear staining within one to two days. The nuclei undergo karyorrhexis and pyknosis.

A secondary factor influencing the staining properties of the involved tissue is the intestinal juices; the intestinal juices must be taken into consideration, for they might loosen the structures and accelerate the disintegration; however, their influence is easily recognized by the uniformity of decolorization and the depth in diffusion, ending in a sharp line without a reactive zone. The picture here does not show such changes; therefore this factor may be disregarded.

A layer of fibrin and leukocytes is in some instances formed on the necrosed loop. It must be assumed that fibrin and leukocytes come from adjacent tissues and are deposited on the serosa, since there is no circulating blood available to act as a source for these blood constituents.

The ligature in the second series was not tied completely and rapidly as in the first but was incompletely and slowly tied so as to permit a low and diminished circulation of blood. The first effect of such a procedure is venous congestion lasting for hours, with visible pulsation of arteries and veins. Dilatation of the intestinal wall follows. To this dilatation and the twisting of the loops must be attributed the final stoppage of the circulation. The twisted loop is black-red, dull, greatly dilated, and filled with bloody fluid mixed with fecal material. The loop stays that way to become enveloped by adjacent loops and omentum until death. Death occurs not later than three days after operation.

The gross picture leaves no doubt that a truly hemorrhagic infarction took place. The microscopic picture is not that of mere congestion with occasional petechiae but that of a uniform heavy hemorrhage into the intestinal wall, identical with that found in the human body in similar cases.

In the experiments recorded here complete and sudden stoppage of the blood supply to the intestinal wall caused anemic necrosis in all the animals without exception. Therefore, there is not an inherent, unexplained and unexplainable tendency of the intestines toward hemorrhagic infarction. The hemorrhagic character of the lesion depends on the special conditions. If the stoppage is sudden and complete, anemic necrosis results; if it is not sudden and complete, hemorrhagic necrosis may result. Now sudden and complete stoppage is practically never realized in a human body. No hernia is incarcerated in a second or at least not completely. It takes time, and half an hour is probably enough to cause venous congestion which slowly aggravates to stasis and infarction. The same is true in cases of volvulus or intussusception. Whether the blood comes from still open vessels or from nearby collaterals can, of course, make no difference. The important factor is the completeness of stoppage of blood supply to the intestinal wall.

The consequences of vascular occlusion on the bowel vary. The superior mesenteric artery may be occluded by thrombosis or embolism without any changes of the intestines (cases have been reported by Dye,[2] McIver,[3] Ross[4] and earlier yet by Virchow[5] and Karcher[6]).

2. Dye, W. J. P.: New England J. Med. **212**:105, 1935.
3. McIver, M. A.: Am. J. Surg. **20**:195, 1933.
4. Ross, G. G.: Ann. Surg. **72**:121, 1920.
5. Virchow, R.: Arch. f. path. Anat. **1**:272, 1847; cited by Warren, S., and Eberhard, T. P.: Surg., Gynec. & Obst. **61**:102, 1935.
6. Karcher, J.: Cor.-Bl. f. schweiz. Aerzte **27**:548, 1897; cited by Dye.[2]

Often at autopsy on persons dying from other causes an embolus is found in this artery without any changes of the bowel. In other cases (2 per cent, according to Trotter [7]) anemic gangrene is found. Still in other cases the changes of the intestine vary from congestion, edema and other reactions to complete infarction and necrosis (McIver [8]).

In 23 per cent of cases Whittaker and Pemberton [9] found arterial occlusion combined with venous thrombosis. The occurrence of venous thrombosis is probably higher than that of pure arterial thrombosis (Warren [10]). The observations in cases of anemic necrosis following arterial occlusion compare closely with these experimental findings.

The cases in which there are no changes are generally explained by collateral vessels.

Cases of arterial occlusion combined with venous thrombosis and with hemorrhagic infarction correspond to the experiments with slow and incomplete occlusion.

The rest, namely, cases of pure arterial occlusion with infarction but with patent veins and collaterals, are still unexplainable.

This much, however, can be drawn from these experiments: 1. Complete and sudden stoppage of the blood supply to the intestines (and probably to any organ) causes anemic necrosis. Hemorrhagic infarction is due to incomplete stoppage. Blood must be allowed afterward to enter the area affected; it makes no difference where this blood comes from—from collaterals, veins or arteries. 2. This blood must enter and remain under sufficiently high pressure. This is realized in cases of venous thrombosis following arterial embolism or in cases of volvulus by venous congestion. Similar conditions exist in cases of incarcerated hernia or intussusception.

The question asked in the introduction as to why in one organ—the lungs or the intestines, for example—a hemorrhagic infarct is usually found, while in other organs—the kidney or spleen—an anemic infarct is regularly found, might now be answered in this way: It is not true that organs differ from one another under the same conditions, but it is true that different organs respond to the same conditions in the same way. In the intestines it has been proved that a complete and sudden stoppage of the blood supply leads to anemic necrosis; the same is true in the kidneys. If in the intestine a hemorrhagic infarct is practically always found, it must be due to special conditions; i. e., the stoppage of the blood supply must be practically never complete and sudden, and there must be pathways left open allowing blood to enter through arteries, veins or collaterals.

7. Trotter, L. B. C.: Embolism and Thrombosis of Mesenteric Vessels, London, Cambridge University Press, 1913; cited by McIver.[3]

8. McIver,[3] p. 196.

9. Whittaker, L. D., and Pemberton, J. de J.: J. A. M. A. **111**:21, 1938.

10. Warren, S., and Eberhard, T. P.: Surg., Gynec. & Obst. **61**:102, 1935.

SUMMARY

The complete and sudden stoppage of the blood supply to the intestines (and probably to any organ) results in anemic necrosis. A hemorrhagic infarction is due to a special condition. Blood must enter the affected area after the initial closure; this blood must come to stay under high pressure. This is realized in cases of combined arterial and venous occlusion, of incarcerated hernia, of volvulus, of intussusception. In none of these cases is there a sudden and complete stoppage of the circulation.

Where the blood in hemorrhagic infarcts comes from seems to be of minor importance. It may come from patent veins or arteries or from collaterals.

It is postulated that the conditions leading to anemic or to hemorrhagic necrosis are the same in any organ.

INCIDENCE AND SIGNIFICANCE OF HEALED MILIARY TUBERCLES IN THE LIVER, SPLEEN AND KIDNEYS

HERBERT S. REICHLE, M.D.
AND
JOHN L. WORK, M.D.
CLEVELAND

The occurrence of small calcified spheres in the liver, spleen and occasionally other of the parenchymatous viscera is well known to pathologists and roentgenologists. Little attention, however, has been given to these lesions in the current medical literature. From time to time they have been referred to as "healed hematogenous tubercles," but more commonly they have been called "phleboliths." [1]

Because of a tentative hypothesis that these bodies are miliary tubercles, a systematic study was conducted on material obtained at autopsy in 500 consecutive cases at the City Hospital, Cleveland, from August 1935 to April 1936. In the search for these lesions, particular care was taken to examine the organs for manifestations of active, latent or healed tuberculosis. Approximately two thirds of the lungs were injected with solution of formaldehyde U. S. P. (diluted 1 to 10) and sectioned. The remaining materials were examined in the fresh state. In a few cases examinations were made with the aid of a fluoroscope, but in the majority the usual methods of palpation and section were depended on to reveal one or more of the components of a primary tuberculous complex, and if they failed to do so the results were recorded as negative.

For purposes of a statistical analysis, partial autopsies and autopsies on stillborn infants, premature infants and infants who died before discharge from the hospital were excluded. There were 48 such cases, leaving a remainder of 452. Nodules were found in one or several of the parenchymatous organs in 91 cases, an incidence of 20.1 per cent for the series. This is a high incidence, since few conditions other than parenchymatous degeneration, hyperemia, edema, primary tuberculosis and arteriosclerosis occur in more than one fifth of the bodies coming to autopsy in this hospital.

From the departments of pathology of Cleveland City Hospital and the Western Reserve University School of Medicine.

1. Moorman, L. J.: Am. Rev. Tuberc. **36**:376, 1937. Berman, T. M.: Radiology **29**:37, 1937. Hellgren, E. G.: Acta pædiat. **13**:180, 1932.

The spleen was most commonly affected. The lesions were found in the spleen in 73 of the 91 cases, in the liver in 47 and in the kidneys in 2. In the liver they were usually situated just beneath but not within the capsule and were only occasionally found in the deeper parenchyma. In the spleen, on the other hand, they were commonly encountered deep in the pulp and infrequently beneath the capsule. The nodules bore no necessary relation to the structure of the organ in which they occurred or to its blood vessels. They could be easily extracted from the parenchyma but could never be shelled out of a casing such as is consistently found about a phlebolith. The number of lesions varied widely from case to case. In a few only one or two were found while in others they numbered several hundred.

The nodules were spherical and varied from 1 to 5 mm. in diameter, with the majority being approximately 2 mm. Occasionally adjacent lesions had coalesced to form lobulated masses, but more commonly they were discrete and widely separated from their neighbors. Grossly they were hard, varied in color from pale yellow to gray and cut with gritty, sometimes bony resistance. When sectioned, they were found to consist of a soft, receding, pale yellow or gray homogeneous center and a thick, pale yellowish gray shell-like periphery. Microscopically (fig. 1) the center was composed of necrotic débris and sometimes a fine fibrillary network with scattered fibroblasts, large mononuclear cells and lymphocytes. In some the center was entirely fibrous. In about half of the nodules the caseous center was surrounded by a ring of hyalinized tissue containing collections of epitheloid cells, which formed radial palisades, lymphocytes and very occasionally a shrunken multinucleated cell. The periphery of the lesion consisted of a thick, extremely well circumscribed capsule of dense collagenous connective tissue which did not contain elastic fibers and which was similar to the specific capsule described by Puhl for the primary tubercle. Very few of the cell nuclei of connective tissue remained, and wandering cells, if present at all, were found only in the outer, looser layers. Varying quantities of calcium were present in the centers or capsules, and in some bone formation had occurred.

Both grossly and microscopically the lesions were indistinguishable from small primary tubercles, i. e., Ghon foci, or from the healed satellite tubercles so frequently encountered in the lungs. They differed from true phleboliths in regard to both position and character and were sufficiently different from small adenomas, fibromas and adrenal rests to prevent confusion with these abnormalities. Because of their gross and microscopic similarity to lesions adequately established as healed tubercles, the assumption that they were monuments to previous tuberculous inflammation appeared justified. Furthermore, an analysis of the data indicates a high positive correlation between the presence

of occult tuberculosis, either healed or active, and the occurrence of calcified nodules in the abdominal viscera. In 86 of the 91 cases (94.5 per cent) in which these nodules were found there was definite healed or active tuberculosis in some other part of the body. In only 5 cases were no tuberculous lesions found and in only 1 of these were roentgen studies undertaken. Further convincing evidence was afforded by the demonstration of tubercle bacilli in these lesions by animal inoculation. Twenty guinea pigs were inoculated with material from the lesions in

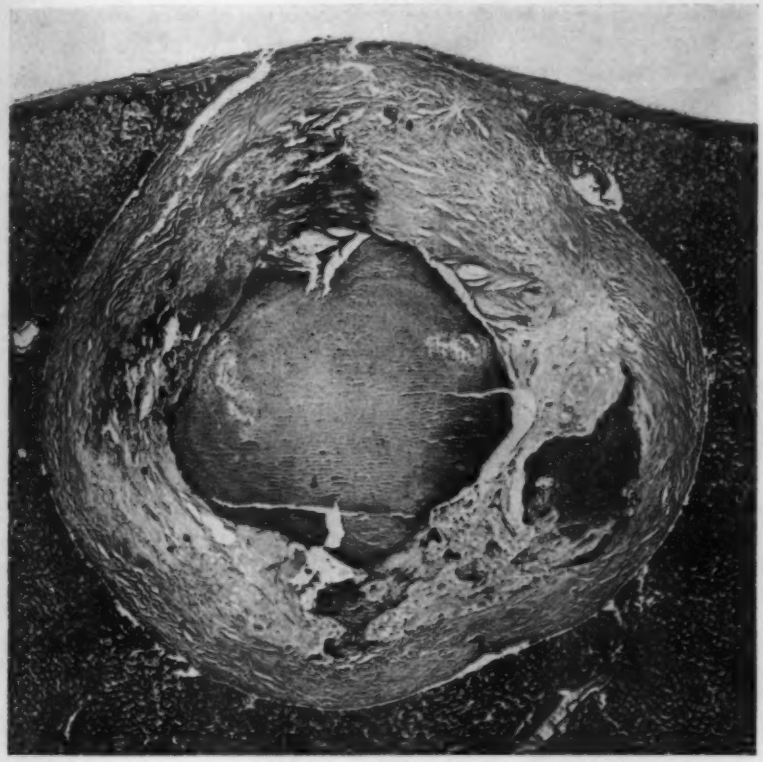

Fig. 1.—Tubercle in liver; hematoxylin-eosin; × 37.5.

14 cases. Precautions were taken to prevent contamination of the material, and no lesion was used if it was associated with active miliary tuberculosis. Because of their small size it was not possible, however, to divide the lesions and use one-half for microscopic study. Positive "takes" were obtained in 4 animals in 3 of the cases.

The morphologic similarity of these lesions to those of healed primary tuberculosis, their concurrence with admittedly tuberculous lesions and the evidence that a fair number of them harbored tubercle bacilli establish them as healed or obsolete tubercles. Their distribution in liver,

spleen and kidneys was such as to indicate that they were the result of hematogenous dissemination. This consideration leads to the following questions:

1. Does hematogenous dissemination have any relation to the development of primary tuberculosis?

2. What effect, if any, does it have on the patient?

It is well known that in large communities most persons who acquire a primary infection do so before the age of 30 years. Therefore, the incidence of these tubercles should be much the same in all age groups over 30 years of age if dissemination occurs early in the course of a primary infection. If, on the other hand, it is a protracted process, occurring from time to time during the lifetime of an infected person, the incidence of healed lesions should bear a direct relation to age. That this is not the case is well shown by figure 4. These lesions therefore developed at a time when the state of reaction characteristic of the primary phase of infection was still active. This constitutes morphologic evidence which demonstrates the probable reality of the second stage in Ranke's classification of the pathogenesis of tuberculosis. Hitherto this stage was regarded as largely hypothetic and as merely bridging the gap between the primary and tertiary stages. In contradistinction to Ranke's opinion, we do not regard stages I and II as sequential; except for the earliest period, which is a matter of days rather than weeks, the two stages run parallel to one another in time. The generalization starts before the primary focus has developed, but the disseminated bacilli, being subject to inherent immunity as well as to the awakening acquired resistance, are reduced in number. The incubation time of primary foci, however, is dependent in a large measure on the number of bacilli. Hence these disseminated foci will develop somewhat later than the primary focus itself.

It is generally accepted that hematogenous dissemination is a common accompaniment of progressive primary tuberculosis and that many of these patients, particularly young children and members of the colored race, die of tuberculous meningitis and generalized miliary tuberculosis. However, there is also abundant clinical, pathologic and experimental evidence that hematogenous dissemination occurs in persons who do not die of the primary infection. One of the cases in our series and another observed subsequently illustrate very well that dissemination can occur early during the course of primary infection. In the first instance a white boy of 10 years died as the result of severe acute streptococcic pansinusitis, bronchitis and mild leptomeningitis, following scarlet fever. At autopsy a typical primary complex was found in the upper lobe of the right lung and in the bronchopulmonary and superior and inferior bifurcation lymph nodes. All parts of the complex showed an exudative type of reaction, and there was no gross caseation of the central por-

tions. In addition, a few active miliary tubercles were demonstrated in the spleen. In the light of previous experience the process was regarded as not older than three months. Nevertheless, there were well developed tubercles in the liver and spleen. In the other case a Negro child of 15 months had been given a clinical diagnosis of tuberculous meningitis, pulmonary tuberculosis and generalized miliary tuberculosis. This child had been exposed to tuberculous disease in the father, who was admitted to the Lowman Pavilion of the City Hospital when the child was only 3 months old. There were no other contacts. The primary focus was located in the upper lobe of the left lung. It had undergone central liquefaction and cavitation and was surrounded by a thick fibrous wall. The lymph nodes were unusually large and caseated. There were a tuberculoma of the brain, tuberculous meningitis and widespread active hematogenous tuberculosis. Of considerable interest was the presence of one perfectly typical healed tubercle in the spleen in addition to active tubercles. The former was calcified and when examined microscopically showed no sign of activity. The observations indicate partial control of a primary infection with subsequent reactivation, widespread dissemination and death. The difference in age of the splenic lesions points to an early as well as a late dissemination. The chronic nature of the pulmonary disease, as well as the healed tubercle in the spleen, clearly indicates that this child must have had tuberculosis for a considerable period and the history establishes the fact that the primary infection occurred some time during the first three months of life. It takes weeks for a primary lesion to develop and months or years for lesions even as small as this to heal and calcify. Therefore the early dissemination presumably occurred between the second and third months of life or within six weeks or two months after the primary infection. These 2 cases conclusively show that dissemination can and does occur very early in the course of the primary disease. This is no new concept of the pathogenesis of human tuberculosis,[2] and it has been shown that bacillemia can exist in experimental animals long before the appearance of a primary lesion. Krause [3] showed that tubercle bacilli may invade the lymphatic and the vascular system within four days of inoculation and become disseminated throughout the body. Willis [4] continued these experiments and came to the conclusion that in guinea pigs inoculated via the skin visceral tuberculosis usually develops even though the area of inoculation is excised within three hours after inoculation. In this respect infection with the tubercle bacillus resembles that with Spirochaeta pallida.[5]

2. Grethmann, W.: Arch. Path. **23**:451, 1937.
3. Krause, cited from Willis.[4]
4. Willis, H. S.: Am. Rev. Tuberc. **11**:439, 1925.
5. Raiziss, G. W., and Severac, M.: Arch. Dermat. & Syph. **35**:1101, 1937.

The significance of this early hematogenous dissemination and of its effect on the infected person was investigated by studying the correlation between the incidence of these healed miliary tubercles and the presence of active tuberculous disease. All cases in which lesions of tuberculosis were shown were divided into three groups according to the apparent resistance which was exhibited to the disease (fig. 2). Into group 1 were placed all cases in which there was neither clinically significant disease nor a lesion showing only microscopically demonstrable activity. The bulk of these cases were those in which only one or more of the components of a primary complex were observed, and

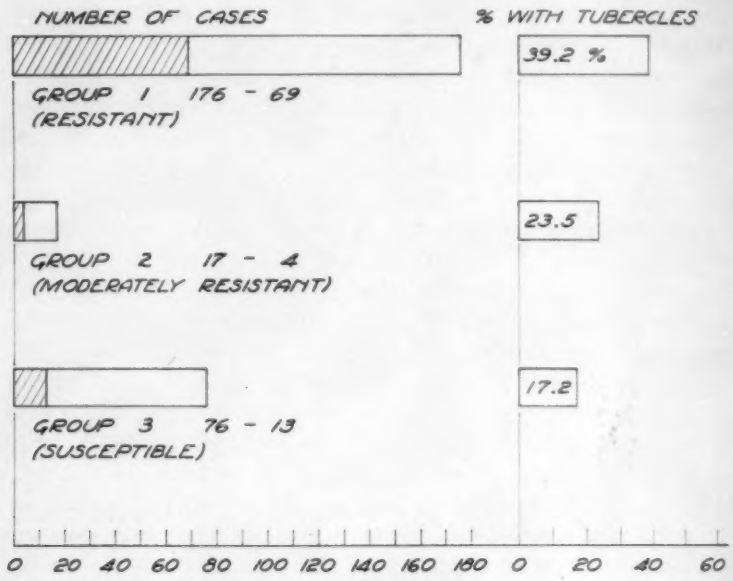

Fig. 2.—Analysis of all cases.

the patients were designated as resistant. Group 2 included cases in which there was no clinically significant disease but in which microscopic sections showed activity; the patients were designated as moderately resistant. Group 3 included only cases in which there was clinically manifest, active disease. In the vast majority of these cases death occurred as a result of tuberculosis. The patients in this group were designated as susceptible. Figure 2 makes it apparent that the incidence of healed miliary tubercles in the so-called susceptible group is only half of that in the resistant. Furthermore, the incidence in the moderately resistant is about midway between that in the resistant and that in the susceptible group. In figure 3 the white persons and the Negroes have been represented separately. Again the highest incidence is found

in the so-called resistant persons of each race and the lowest incidence is in the susceptible. The number of cases in group 2 is probably too small to be of any statistical significance, yet, as in figure 2, the percental values are intermediate between the other two groups. No adequate explanation for the differences between the two races is offered. Figure 4, referred to earlier in this paper, shows that there is no correlation between the chosen age groups and the incidence of healed miliary lesions in the spleen and liver. In spite of the small numbers in some of the groups, the differences already noted between the susceptible and resistant groups are still apparent.

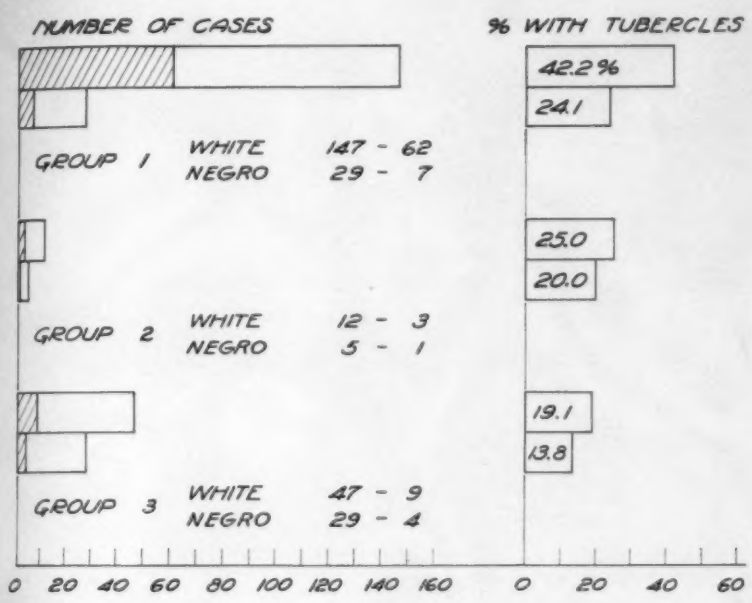

Fig. 3.—Analysis by race.

From these diagrams several valid conclusions can be drawn. First, there is no correlation between age and incidence of healed miliary tubercles. Second, the percental incidence of healed tubercles is greater in the white than in the Negro race. Third, the incidence of healed tubercles is greater in persons who, on the basis of anatomic findings, have been considered resistant than it is in more susceptible persons, and these differences are constant for both races. For this there are at least two possible explanations. One is that a large number of the more susceptible succumb to the infection at the time of the early hematogenous spread. If this were the correct and only explanation, however, it might be expected that, if all persons dying of progressive primary tuberculosis, as well as persons dying from other causes with incidental

active primary lesions, were deducted from the total number of patients of group 3, the percental incidence of healed miliary tubercles computed on the basis of the corrected figure would approximate that of the first group. There were only 2 such persons in group 3, and both of these were children who died of causes other than tuberculosis and presented at the time of autopsy active primary complexes as well as active miliary tubercles in the spleen. With these 2 patients deducted from group 3 the incidence of healed tubercles is raised to only 18 per cent, a figure which is still significantly different from 39.2 per cent, the incidence in group 1. The second explanation for this difference is that an early hematogenous spread, with limited seeding of tubercles in the organs,

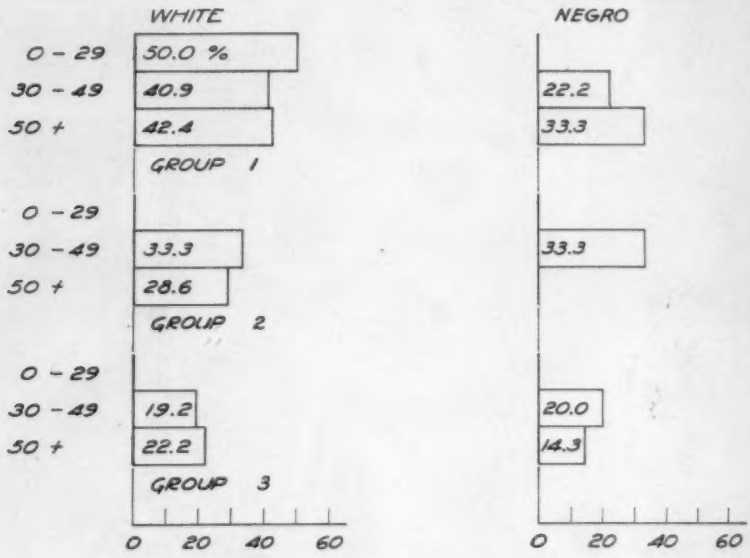

Fig. 4.—Percental analysis of cases by race and age.

acts as an "autovaccination" and confers protection against reinfection. That the spread of a disease should serve to check the progress of that disease at first appears to be a paradox. It is, however, well known that this does occur in tuberculosis. Persons with extensive pulmonary disease rarely die of miliary tuberculosis, and, on the contrary, pathologists have long known that typical acute miliary tuberculosis of the adult is usually associated with some relatively unimportant form of organ tuberculosis.

Had accurate counts of the number of tubercles present in the liver, spleen and other organs been made in each case, it is possible that the figures thus obtained would have served as rough indexes of the degree or intensity of the dissemination. And, if so, a positive correlation

between the number of tubercles and the manifest resistance might be expected. This aspect of the problem was not appreciated until it was too late to make such counts. A review of the lung-diagrams made at the time of autopsy, however, suggests that dissemination was generally more widespread in the relatively resistant than in the relatively susceptible. In 22 of the 69 cases in group 1, or 31.9 per cent, there were calcified tubercles in the lung parenchyma other than the primary focus. This compared with 1 of 4 (25 per cent) and 1 of 13 (7.7 per cent) in groups 2 and 3, respectively.

SUMMARY

Many, if not all, of the small spherical bodies found in the liver, spleen and kidney and frequently called phleboliths are true miliary tubercles. They occur in approximately one fifth of the bodies coming to autopsy at the City Hospital, Cleveland. They are histologically indistinguishable from small primary tubercles. Animal inoculation has demonstrated the presence of tubercle bacilli. Their distribution points to hematogenous dissemination and their morphology to some association in point of time with the primary complex. The high correlation between apparent resistance to progressive tuberculous disease and the incidence of these lesions leads to the suggestion that this dissemination acts as autovaccination, conferring a relatively high resistance to reinfection.

ACTION OF ESTROGEN ON SKELETAL TISSUES OF IMMATURE GUINEA PIGS

MARTIN SILBERBERG, M.D.
AND
RUTH SILBERBERG, M.D.
ST. LOUIS

In previous investigations [1] we found that gonadectomy had a growth-promoting effect on the euhyaline cartilage of the immature guinea pig. Differences in the response of the sexes were observed: The proliferation was somewhat more marked in male animals; in these, likewise, the balance between proliferation, on the one hand, and calcification and ossification, on the other, was more disturbed. In pursuance of these investigations we thought that a study of the action of estrogen might give further insight into the role of the gonads in the processes of growth and ossification of cartilage.

MATERIAL AND METHODS

Forty-eight guinea pigs, born in the spring, were used; 24 of these animals were given an intraperitoneal injection of 20 rat units of estrogen dissolved in 0.9 per cent saline solution, six times weekly; 24 others were given each a dose of 250 rat units of estrogen in oil subcutaneously, twice weekly. In each of these two groups 12 animals were males and 12 were females; at the beginning of the experiments one half of the guinea pigs weighed between 140 and 160 Gm. and the remaining half between 180 and 190 Gm. The large majority of the animals were killed three, seven, fourteen, twenty-one, thirty and sixty days after the injections were started, but 3 guinea pigs had to be killed for various reasons at intermediate times.

At autopsy, one tibia, the knee joint of the other side, ribs and vertebrae were removed for study and subjected to the same technical procedures as previously described.[2]

CHANGES IN WEIGHT

The variations in the weights of the animals were similar in the different groups. Slight differences became apparent only in those

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

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1. Silberberg, M., and Silberberg, R.: Am. J. Path. **15**:55, 1939.
2. Silberberg, M., and Silberberg, R.: Arch. Path. **26**:1208, 1938.

animals in which the injections of estrogen were continued for two months. In table 1 the initial and final weights of these animals are given, the first two horizontal rows representing the weights of the guinea pigs which weighed about 150 Gm. at the beginning of the experiment, and the last two horizontal columns the corresponding figures for the animals which had initial weights of about 180 Gm. The table is divided into two sections, one for the males and the other for the females. Each section is subdivided into three vertical columns. The first two show the weights of the normal controls, the second the mean weights of the guinea pigs given injections of the watery solution of estrogen, and the third row the mean weights of the animals which received the oily solution of estrogen.

Total amounts of 1,040 rat units of estrogen in saline solution and 4,000 rat units of estrogen in oily solution were administered.

TABLE 1.—*Variations in Weights of Animals During the Experiments*

	Female Animals			Male Animals		
	Normal	Estrogen in Saline Solution, 1,040 Rat Units	Estrogen in Oil, 4,000 Rat Units	Normal	Estrogen in Saline Solution, 1,040 Rat Units	Estrogen in Oil, 4,000 Rat Units
Light animals:						
Initial weight.....	154	148	153	147	153	149
Weight after 60 days..	385	375	365	393	425	320
Heavy animals:						
Initial weight.....	182	182	181	180	180	178
Weight after 60 days..	410	380	355	395	435	370

MICROSCOPIC OBSERVATIONS

Epiphysial Line.—As early as three days after the beginning of the injections, but more definitely after one week's treatment, the zones of endochondral ossification became narrower than ordinarily, and they revealed a slightly increased tendency to calcification.

The first changes observed in the intercartilaginous chondromucoid ground substance were of a retrogressive character. They consisted of a loosening of the matrix, which became vacuolated and later dissolved. The degenerative processes did not spread evenly throughout the epiphysial line but formed circumscribed, chiefly wedgelike lesions, the base of which was located at the proximal part of the epiphysial line. These alterations usually occurred after from 240 to 500 rat units of estrogen had been administered. With increasing doses of estrogen and increasing duration of the experiment, the degenerative processes took one of two courses: They became intensified and affected larger areas, which ultimately underwent calcification and finally ossification, or they remained more localized, and the areas thus affected became converted

into collagenous hyalinized material which at later stages became ossified without preceding calcification. As a rule, the larger the amounts of estrogen given and the longer the time during which the substance acted, the more advanced was the ossification of the intercartilaginous matrix.

In order to determine more exactly the width of the epiphysial line, a count of the cartilage cells in the epiphysial line of the upper end of the tibia was made, as in our former investigations. The average

TABLE 2.—*Ratio of Numbers of Hypertrophic and Columnar Cells in One Row in the Epiphysial Line of the Tibia as Observed in Guinea Pigs Treated with Estrogen for Varying Periods: Smaller Doses*

Period, Days	Total Dose of Estrogen Administered, Rat Units	Light Animals		Heavy Animals	
		Males	Females	Males	Females
3	60	4:10-11	4:9	4:10	4:10
7	120	4:10-9	3-4:7-8*	4:9-10	4:9-10
14	240	4:13-14†	4:13	4:16-17	4:12-13
21	360	2:14-15	0-2:14-15	0-3:14-15	3-4:14
30	520	4:15	3-4:12-13	2-3:15-16	3:14-15
60	1,040	4:16	3-4:8-9	3-4:14-15	2-3:6-7

* When this guinea pig was killed, it had received 160 rat units.
† When this guinea pig was killed, it had received 300 rat units.

TABLE 3.—*Ratio of Numbers of Hypertrophic and Columnar Cells in One Row in the Epiphysial Line of the Tibia as Observed in Guinea Pigs Treated with Estrogen for Varying Periods: Larger Doses*

Period, Days	Total Dose of Estrogen Administered, Rat Units	Lighter Animals		Heavier Animals	
		Males	Females	Males	Females
3	250	4:10	4:8-9	4:9	3-4:7-8
7	500	4:6	4:8-9	4:8	4:8-9
14	1,000	2-3:14-15	2-3:6-7	2-3:16-17	2-3:13-14
21	1,500	2-3:7-8	2-3:6-7*	2-3:7-8	2:6
30	2,000	3-4:14-15	3:7-8	3-4:9-10	4:15-16
60	4,000	4:8	4:8-9	3:8-9	4:7-8

* When this animal was killed, it had received 1,250 rat units.

number of hypertrophic and of columnar cartilage cells in one row and the ratio between these kinds of cells were established. In normal guinea pigs of corresponding weights, on the average, 4 hypertrophic cartilage cells and 10 columnar cartilage cells were found in one cell row.

Tables 2 and 3 show the individual cell counts and the ratio between the numbers of hypertrophic and columnar cartilage cells after the administration of estrogen. In table 2 are given figures obtained following the injections of the smaller doses of estrogen, and in table 3, those obtained after the injections of the larger doses.

As seen in tables 2 and 3, the narrowing of the epiphysial line in the early stages was due in some instances to a decrease in the number of the columnar cartilage cells. In animals treated with 500 rat units of estrogen in an oily solution the number of the columnar cartilage cells could be as low as 6 to 8, whereas in those treated with 120 rat units of estrogen in a saline solution over the same period there could be a very slight decrease in the number of the columnar cells. The hypertrophic cartilage cells did not show any definite change at this period. After approximately fourteen days a reverse process had set in (fig. 1 *A*).[2a] The epiphysial line had become distinctly widened. A differential count of the hypertrophic and columnar cartilage cells revealed that this enlargement of the epiphysial line was due to an increase in the number of the columnar cartilage cells; the hypertrophic cartilage cells had become less numerous. The number of columnar cartilage cells in one cartilage row was in some instances as high as 16; the hypertrophic cartilage cells, on the other hand, were not infrequently reduced to 2 or to 1, and in some places they were entirely lacking. In most cases the diminution in the latter type of cells occurred in those guinea pigs which had received estrogen in saline solution over long periods. This diminution was found in some animals even after treatment for as long a time as two months, when approximately 1,000 rat units in a watery solution had been injected. Those guinea pigs, however, which had been given the larger doses of estrogen in an oily solution showed this enlargement of the epiphysial line in the great majority of cases for a shorter period of two weeks only. This also coincided with the time when about 1,000 rat units had been administered. In 2 instances, however, an enlargement of the epiphysial line was found after a total dose of 2,000 rat units of estrogen in an oily solution had been injected over a period of one month. The second narrowing (fig. 1*B*) of the epiphysial line in the animals which had received more than 1,000 rat units of estrogen in a watery solution took place in the female animals after a period of two months, at a time when, in the corresponding males, the epiphysial line was still wide. A very definite second narrowing of the epiphysial line was found in 6 of 8 animals which had received 1,500 and 2,000 rat units of the oily solution over periods of three or four weeks, and 4,000 rat units of the oily solution, injected over a period of two months, caused a narrowing of the epiphysial line in all instances.

Associated with these changes in the numbers of cartilage cells were certain other changes which affected to some extent also the resting cartilage but concerned mainly the columnar and hypertrophic cartilage.

2a. Mr. S. J. Hayward made the photomicrographs.

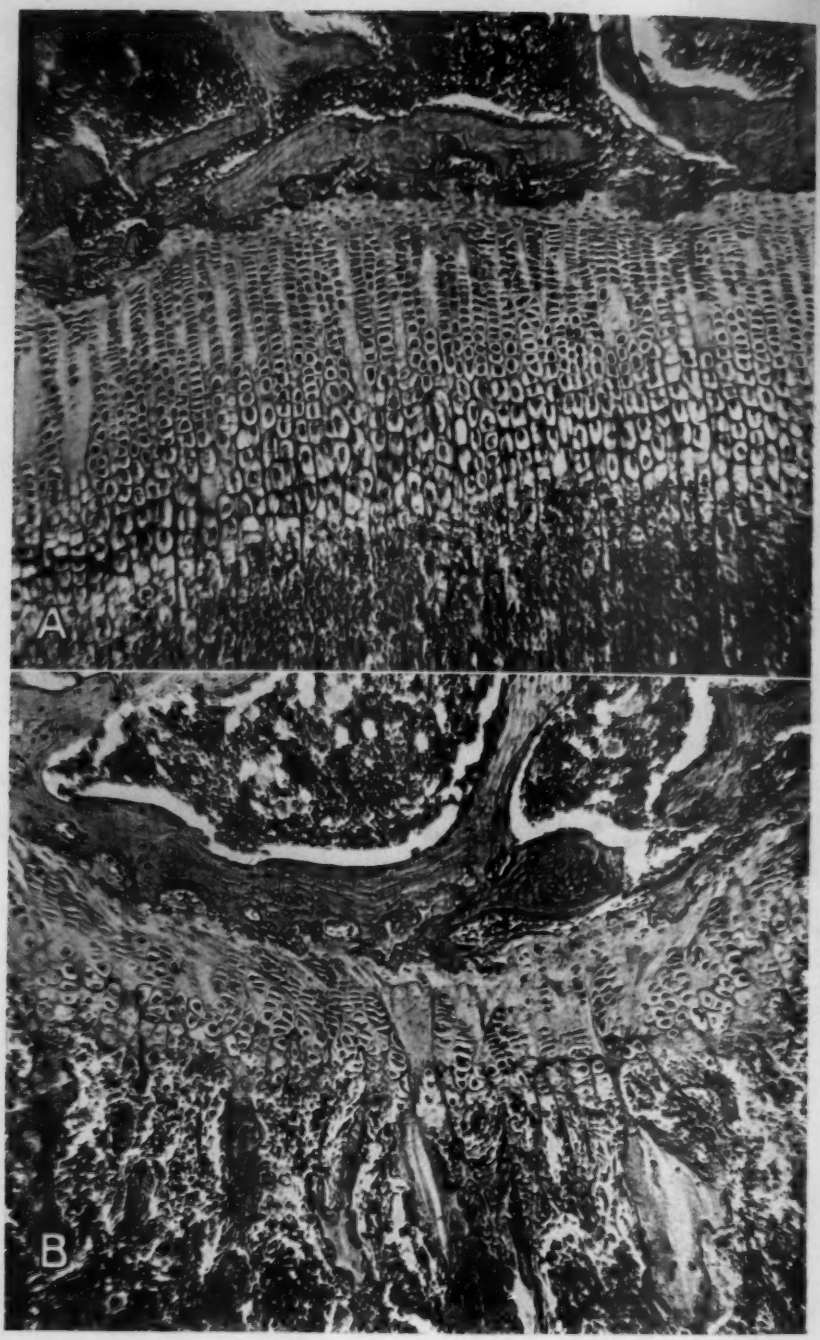

Fig. 1.—*A*, photomicrograph of a section of the epiphysial line of the tibia of a female guinea pig which had received 240 rat units of estrogen in a watery solution over a period of two weeks; × 144.8. The weight of the animal increased from 180 to 240 Gm. The epiphysial zone is enlarged. The columnar cartilage cells proliferate. The intercartilaginous matrix is swollen in some areas.

B, photomicrograph of a section of the epiphysial line of the tibia of a female guinea pig which had received 1,040 rat units of estrogen in a watery solution over a period of two months; magnification, same as in *A*. The weight of this animal increased from 185 to 380 Gm. The epiphysial zone is narrowed. The columnar cartilage cells are diminished in number. The cartilaginous rows are irregular and in some places lacking. The intercartilaginous matrix is increased in amount and sclerosed.

1. *Resting Cartilage.* In those cases in which a decrease in the number of proliferating cartilage cells was seen, the resting cartilage cells remained inactive. However, with an increase in the number of columnar cartilage cells the zone of the resting cartilage appeared likewise activated. More cells were formed, the darkly stained nuclei assumed an elongated or oval shape, the cytoplasm became irregular, enlarged and thin, and branched processes were sent out by these cells.

2. *Columnar Cartilage.* In the cases in which a decrease in the number of cells had occurred the alterations were different from those in cases in which a proliferation of these cells had taken place. In the former, the cartilage cells lying in one column had regular outlines, their cytoplasm was narrow, and it contained a dense, rather flattened nucleus. In contradistinction to the normal condition, in which the transition from columnar into hypertrophic cartilage takes place by a gradual increase in the size of nucleus and cytoplasm, transitional cell forms were lacking here. However, in individual instances the intensity of these changes varied. In those instances in which the cells multiplied markedly, some cells showed progressive hydropic swelling of the cytoplasm (fig. 2*B*), and, owing to the increased pressure within the cells thus produced, the nuclei were pushed toward the periphery, so that not infrequently seal ring cells were formed. Further retrogressive changes led then to complete degeneration and destruction of the cells. In advanced cases whole cell rows were not only injured but in some instances entirely destroyed. This condition, to which were added degenerative processes in the intercartilaginous ground substance, caused irregularities throughout the epiphysial line. However, in other instances such degenerations did not occur; instead, the nuclei increased in size and rounded off, and the cytoplasm likewise appeared enlarged. The columnar cells then assumed a spheroid shape, and more and more they tended to take acid stains. At this stage they underwent frequent mitotic division (fig. 2*B*). The maximum of nuclear division was seen when the animals had a weight of from 225 to 275 Gm. Mitoses were found more frequently in the proximal part of the rows of cartilage cells; in the majority of animals they were numerous in the center of the epiphysial line and somewhat rarer toward the peripheral areas. In the beginning, as a rule, the 2 daughter cells occupied the original space of the mother cell and were separated from the upper and lower neighboring cells of the row by a thin but distinctly visible network of intercellular substance. Later on, with further increase in number and size of cells, not only 2 but sometimes as many as 10 and more such acidophilic cells were packed together in one alveolar space, in which no intercartilaginous ground substance was seen.

At still later periods, both the proliferative and the retrogressive changes in the cartilage gradually receded, and then one of two ways

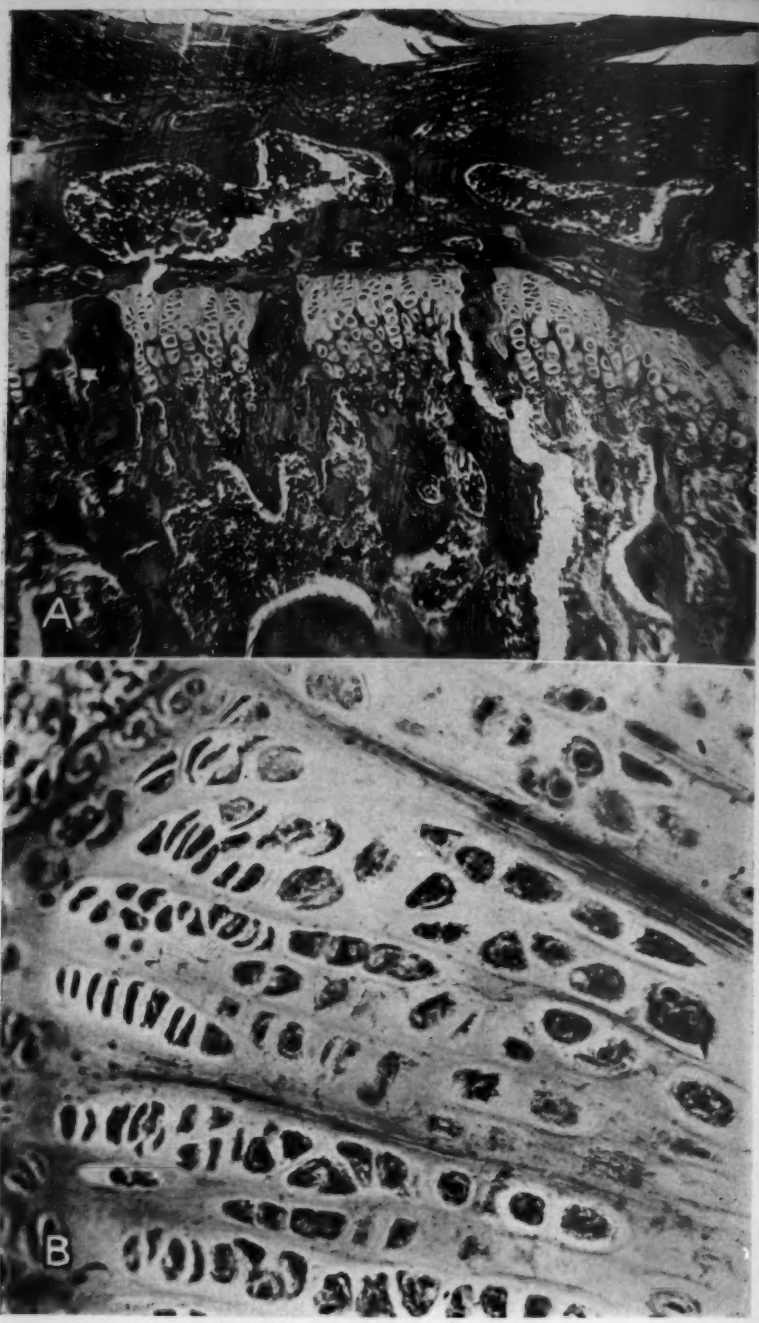

Fig. 2.—*A*, photomicrograph of a section of the epiphysial line of a vertebra of a female guinea pig which had received 1,040 rat units of estrogen in a watery solution over a period of two months; magnification, same as in fig. 1 *A*. The weight of the animal increased from 150 to 375 Gm. The epiphysial zone is narrowed. Large osseous masses which unite the diaphysial and epiphysial bones have replaced the former disintegrated cartilaginous rows. Three osseous plugs are seen in the epiphysial zone.

B, photomicrograph of a section of the epiphysial line of the tibia of a female guinea pig which had received 360 rat units of estrogen in a watery solution over a period of three weeks; × 326.6. The weight of the animal increased from 155 to 250 Gm. Some normal columnar cartilage cells are still visible in the left part of the picture. The majority of the cartilage cells have undergone proliferation, hypertrophy and degeneration. Seal ring cells and cells of epithelioid structures have been formed. Mitosis is noted in the center. The intercartilaginous matrix is increased in amount and shows fibers running in a parallel direction, resembling "asbestos transformation."

of reaction could be distinguished: (*a*) The cell capsules became thickened, and at the periphery of the basophilic cells an intensified incrustation with calcium salts was noticeable, which latter subsequently penetrated into the cells. The arrangement of the rows remained irregular. Thus, under the influence of estrogen, in animals approximately 3 months old and weighing between 350 and 425 Gm. the columnar cartilage resembled in histologic appearance in some cases that of normal guinea pigs weighing 700 to 800 Gm. and approximately 8 to 9 months old. Or (*b*) the columnar cartilage cells did not undergo calcification and stained markedly with acid dyes. Then, the ossifying matrix began to enclose the cells, and gradually a direct conversion of the cartilage cells into preosteocytes and osteocytes took place. Osseous plugs were formed which extended longitudinally through the epiphysial zone. In the center of these plugs preserved cartilage cells of columnar type could still be recognized. Such cells were also occasionally found in newly formed trabeculae which reached downward into the bone marrow. In these stages the epiphysial line appeared not unlike that of normal animals at the end of the first or in the second year of life.

3. *Hypertrophic Cartilage.* The reduction in the number of hypertrophic cartilage cells was due to an accelerated breakdown of the cells and their replacement by bone, and it was accompanied by intensified calcification and ossification of the intercartilaginous matrix. If calcification predominated, capillaries of the bone marrow eroded the capsules of the cartilage cells, and numerous epithelioid cells and multinucleated phagocytic giant cells advanced into this region. Subsequently, a quick and accelerated substitution of the calcified cartilage by bone took place. The greater the rapidity of ossification in comparison with the speed of transformation of the columnar into hypertrophic cartilage, the narrower became the layer of hypertrophic cartilage. In cases, however, in which the calcification of the hypertrophic cartilage was less marked, the cartilage cells were not broken down, but their direct conversion into osteocytes was observed, a phenomenon analogous to that described in the columnar cartilage. In contrast to the evenly progressing process of replacement, metaplasia of cartilage into bone occurred in an irregular manner in various areas, particularly where thick collagenous acidophilic substances had been deposited in the epiphysial line. It seemed to be the contact of the cells with this collagenous material which gave rise to different centers of ossification. Such centers coalesced in places with the ossified areas in the columnar zone and helped to form the aforementioned osseous plugs (fig. 2*A*).

Chondrophyte.—Conditions here correspond to the changes which took place in the epiphysial line. In those instances in which hyperplasia

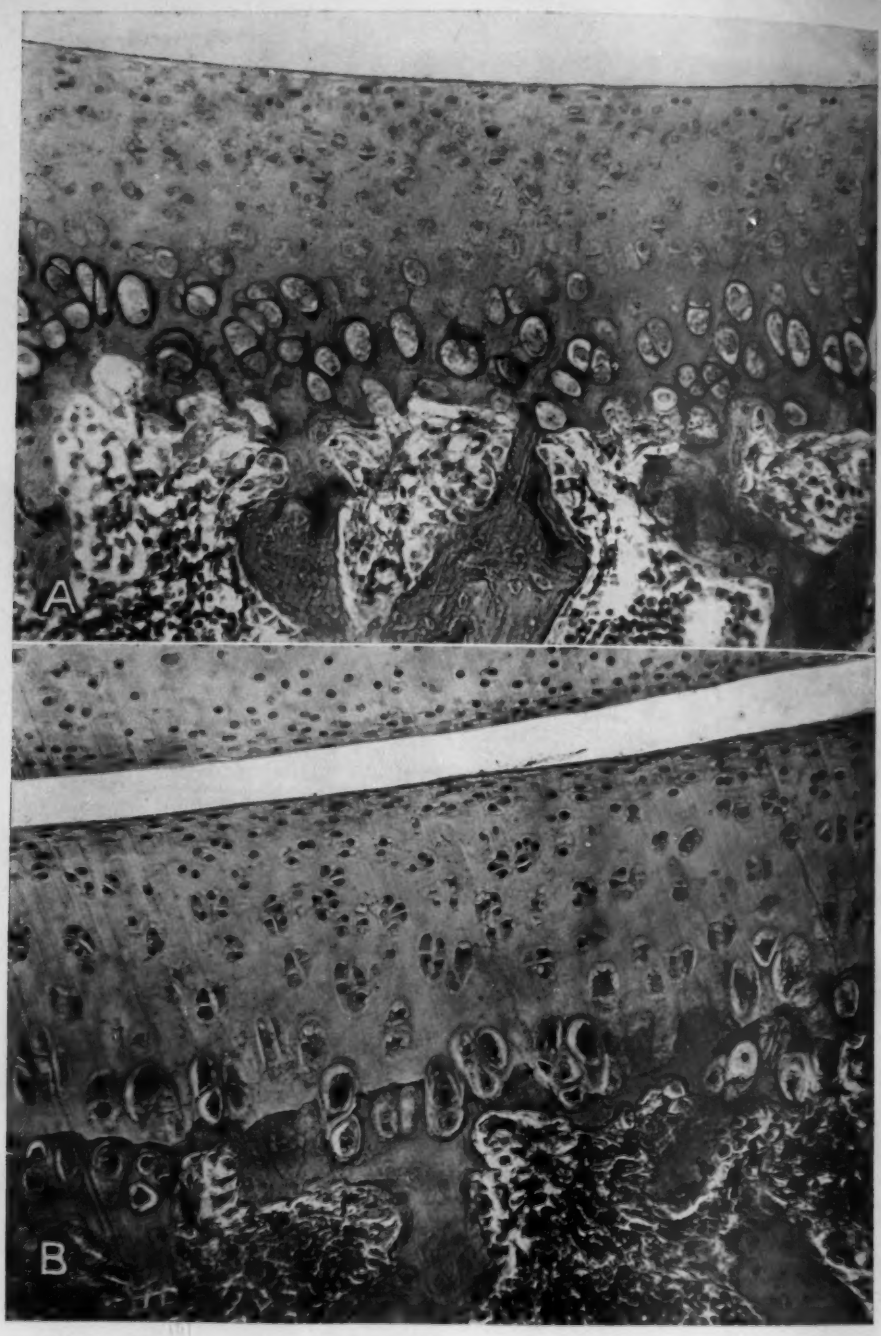

Fig. 3.—*A*, joint surface of the tibia of a normal female guinea pig; × 220. The weight of the animal was 200 Gm. The cells of the various cartilage zones are well preserved. The uppermost or sliding zone shows flat resting cells horizontally arranged. The cells of the transitional zone are somewhat rounded off and likewise resting. The cells of the pressure zone show a fairly regular arrangement. Hypertrophic cartilage cells are present in normal number. Ossification is normal.

B, joint surface of the tibia of a female guinea pig which had received 360 rat units of estrogen in a watery solution over a period of three weeks; magnification, same as in *A*. The weight of the animal increased from 155 to 230 Gm. Ossification is advancing and engulfing many hypertrophic cartilage cells. Cells of the pressure and transitional zones hypertrophy and some of them degenerate. Cells of the sliding zone are somewhat hyperplastic. The intercartilaginous stroma is increased in amount and hyalinized.

and hypertrophy particularly of the resting epiphysial cartilage were noted, a slight hyperplasia and hypertrophy of the euhyaline cartilage were also seen in the lateral protuberances, especially in places adjoining the epiphysial line. If the proliferation was very much accentuated, slight retrogressive changes could be detected not only in the cartilage cells but also in the interstitial substance.

Joint.—After one week's treatment the zone of hypertrophic cartilage was already found to be narrowed, provided as much as about 500 rat units of estrogen had been injected during this period. The intercartilaginous matrix increased more and more; it became sclerosed and at later periods osseous. In contradistinction to the normal processes (fig. 3*A*), in which the cartilage cells broke down by way of chondroclasis and subsequently were replaced by bone, in the estrogen-treated guinea pigs not infrequently a direct conversion of the hypertrophic cells into osteocytes occurred. Thus hypertrophic cartilage cells surrounded by bony substance were observed not only in the ossifying layer but also in the trabeculae of the epiphysial cavity. The bony border lamella which demarcates the zone of hypertrophic cartilage was distinctly thickened (fig. 3*B*), and gradually the ossification began to advance toward the pressure and transitional zones. At this stage the cartilage cells of these layers became more numerous and enlarged. One cartilage capsule often contained as many as 4 or more large cells, thus indicating that proliferation had taken place. In other instances retrogressive changes were noticeable. The enlarged cytoplasm then became swollen, vacuolated and liquefied; karyorrhexis and karyolysis were frequently seen. The farther the ossification proceeded, the more pronounced became both progressive and retrogressive changes in the cartilage. Within two to four weeks, and this was especially the case in the guinea pigs treated with the larger amount of estrogen in oily solution, thick osseous masses replaced the original hypertrophic and pressure zones. Simultaneously, the normally flat and horizontally arranged cells representing the transitional zone took on a perpendicular arrangement and reacted in the same way as the cells of the original pressure zone. They multiplied and hypertrophied. Slight to moderate proliferation of the cellular constituents of the sliding zone was found in some instances (fig. 4). The cartilage of the female guinea pig responded more readily and more intensely to the action of estrogen than that of the male under corresponding conditions. In the males hyperplasia and, in particular, also hypertrophy were less accentuated than in the females.

After the large doses of estrogen had acted for a period of two months, cellular proliferation was no longer seen; however, pronounced thickening of the cortex of the joint was visible. This consisted of

a dense layer of bone lying underneath a narrow sheet of cartilage, which was the remnant of the former covering. Inside the osseous masses the original line of calcification was still present in some places.

Bone Marrow.—At the earlier stages of the injections of estrogen the marrow contained more fat cells than ordinarily. Chiefly near the endosteum and in the neighborhood of the bony spiculae in the subepiphysial layer, the connective tissue became thickened by the deposition of a larger amount of collagenous fibers, which later under-

Fig. 4.—Joint surface of the tibia of a female guinea pig which had received 4,000 rat units of estrogen in an oily solution over a period of two months; magnification, same as in figure 3*A*. The weight of this guinea pig increased from 150 to 365 Gm. Ossification is far advanced; it has entirely replaced the hypertrophic zone and partly replaced the pressure zone. The cells of the transitional zone are markedly hyperplastic and hypertrophic and show a perpendicular arrangement. Some cells have undergone degeneration. Cells of the sliding zone have partly rounded off. The intercartilaginous matrix contains large masses of sclerosed material, which produce the spotty and uneven appearance of the ground substance in the picture.

went hyalinization and sclerosis. The fibrocytes proliferated markedly, and there were many mitotic figures noticeable. At the same time the mesenchymal reticulum showed accelerated development in the

direction of (*a*) fibrosis or (*b*) production of bone-forming elements or (*c*) production of megakaryocytes.

The fibrous changes in the reticulum intensified the fibrosis in the marrow. In some instances the mesenchymal spindle cells rounded off, and numerous mitoses appeared in these small to medium-sized ameboid acidophilic epithelioid cells. By accumulation of such cells an osteoid tissue developed. This took place particularly at later stages in the subepiphysial areas adjoining the layer of hypertrophic cartilage of the epiphysial zone and of the joint, where in the beginning a fibrous tissue had been laid down. In other places these cells did not assume a tissuelike structure but were arranged in a beadlike manner. This could be observed particularly around the bony trabeculae. In some cases the epithelioid cells acted as osteoblasts which underwent further differentiation into osteocytes; in other instances they coalesced, producing osteoclastic phagocytic giant cells which absorbed and caused the solution of the hyalinized and osseous masses, which latter had been produced in excessive quantities. In the earlier stages of the experiments the appositional processes predominated, as a result of which the number of trabeculae became increased and the individual trabeculae were thickened in the subepiphysial zone. After one to two months' treatment with estrogen, however, the resorptive processes were comparatively more accentuated. Thus more normal conditions were restored, and in some cases the trabeculae seemed even to be thinner than under ordinary conditions. Farther distally the alterations in these structures were much more moderate, although they were similar in character; near the endosteum, fibrous changes were quite marked. At the end of two months of treatment with estrogen there was no evidence of apposition of bone at the endosteum.

As far as the effect on the megakaryocytes is concerned, the hemocytoblasts were pronouncedly increased in number after three to four weeks of estrogen administration. They frequently underwent fusion and nuclear division. Numerous megakaryocytes were formed, which showed ameboid activity and multiplied mitotically. They were accumulated chiefly in the neighborhood of the trabeculae, where they were held back and at later stages could not be distinguished from the giant cells of epithelioid character.

The question arises as to whether the increase in the number of the megakaryocytes was due solely to proliferation of the cells, or whether it went hand in hand with increased resistance of these young megakaryocytes to injurious conditions and decreased tendency toward disintegration. The latter assumption must be taken into serious consideration, especially in view of the observation made more recently that after administration of estrogen a decrease in number of thrombocytes takes place in the peripheral blood and that severe spontaneous

hemorrhages have been reported in dogs following this decrease in thrombocytes (Arnold and co-workers [3]; Schrade [4]). In one of our animals which had to be put to death we found an extensive spontaneous subphrenic hemorrhage, which may perhaps have been due to thrombopenia.

As described, in the epiphysial line some trabeculae might still contain single cartilage cells or groups of cartilage cells, either of the hypertrophic or of the columnar type, which had been directly converted into osteocytes; intensive ossification, however, had taken place in 46 of 48 cases; in only 2 instances the formation of strands of hypertrophic calcified but not as yet ossified, or only incompletely ossified, cartilage was suggested. In these instances we had, however, to deal with abnormal conditions. One animal had died of peritonitis; the other had had an attack of pneumonia and had suffered loss of weight. These 2 cases have necessarily to be discarded for the purpose of analyzing the effects of estrogen on cartilage and bone.

Bony Shaft.—The first changes were seen after one week's treatment and were more marked when higher doses of estrogen had been given. The periosteal connective tissue became denser, thickened and sclerosed; it increased gradually in amount and consisted of masses of collagenous and hyalinized fibers, among which were numerous fibrocytes which had proliferated by way of mitoses. On the other hand, the osteoclastic giant cells were definitely diminished as compared with the normal conditions, and they were separated from the bone by a layer of fibrous connective tissue. Therefore the surface of the compact bone was smoother than is ordinarily the case, and only a few, quite superficial lacunar grooves were visible. This was the state of the periosteum at the period when appositional processes predominated along the trabeculae in the bone marrow. At later periods there appeared within the periosteal connective tissue, medium-sized to large round epithelioid acidophilic cells, which in some areas were arranged in small islets and longitudinal groups and in other places had coalesced and formed giant cells. One has the impression that the accumulations of such cells were the consequence of the proliferation and hyalinization of the connective tissue. The latter processes inhibited the ameboid movement of the epithelioid cells, which were held back and could not come in contact with the compact bone.

After administration of estrogen for periods of one month and over, large round and spindle-shaped cells, representing various transitional structures between fibrocytes, precartilaginous cells and

3. Arnold, O.; Hamperl, H.; Holtz, F.; Junkmann, K., and Marx, H.: Arch. f. exper. Path. u. Pharmakol. **186**:1, 1937.

4. Schrade, W.: Folia haemat. **61**:145, 1939.

typical euhyaline cartilage cells, were found in the periosteal connective tissue. The closer these cells were situated to the compact bone, the more they enlarged and took on the definite shape of cartilage cells, in which the cytoplasm often was very much vacuolated (fig. 5*A*). In some places, chiefly in the middle of the shaft, such cartilage cells were found within the osseous masses; they seemed to have invaded and dissolved the compact bone. Between these cells there remained large strands of dense acidophilic substances. The cases in which these marked processes of invasion and solution of the bone took place were those in which also around the trabeculae of the marrow the resorptive changes were more pronounced than the appositional ones. The development of cartilage cells along the shaft was found in all the 8 animals which were examined after two months of treatment with estrogen and in the majority of those examined as early as after one month. Although in some instances the haversian channels seemed to be less frequent than ordinarily, no evidence of an appreciable apposition of bone could be detected after two months' treatment.

Ribs.—In the cartilaginous part of the ribs the resting cartilage degenerated in direct proportion to the amount of estrogen administered and the time during which it acted. The cytoplasm of the cells appeared enlarged and vacuolated. The nuclei became pyknotic and finally disintegrated. Thus changes which under normal circumstances are seen in old guinea pigs became more and more intensified. In the beginning, only single cells were affected; but later whole groups of cells underwent this retrogression. The intercartilaginous matrix became first swollen and vacuolated; at later stages it increased in amount, and especially around the cytoplasm of the degenerating cells acidophilic collagenous and hyalinized substances were seen covering circumscribed areas and including more or less preserved cartilage cells or cell débris (fig. 5*B*). In cases in which the process was advanced, such sclerosed material resembled osseous ground substance, although the enclosed cells could still have the characteristics of cartilaginous elements. This condition differed from that seen in normal old guinea pigs, inasmuch as under the influence of estrogen hyalinization of the degenerated cartilage occurred, whereas calcification of the degenerated areas was lacking. In the zone of proliferating cartilage the matrix and the cells occasionally underwent similar, although less marked, retrogressive alteration, but at later periods, especially, growth processes became noticeable. The columnar cells multiplied frequently by way of amitotic division. The layer of the hypertrophic cartilage was somewhat narrowed, and the cells showed only a moderate degree of calcification. On the other hand, ossification proceeded with the same rapidity and intensity as in the epiphysial line of the long bones. At the periosteum, chiefly near the chondro-osseous junction, thickened

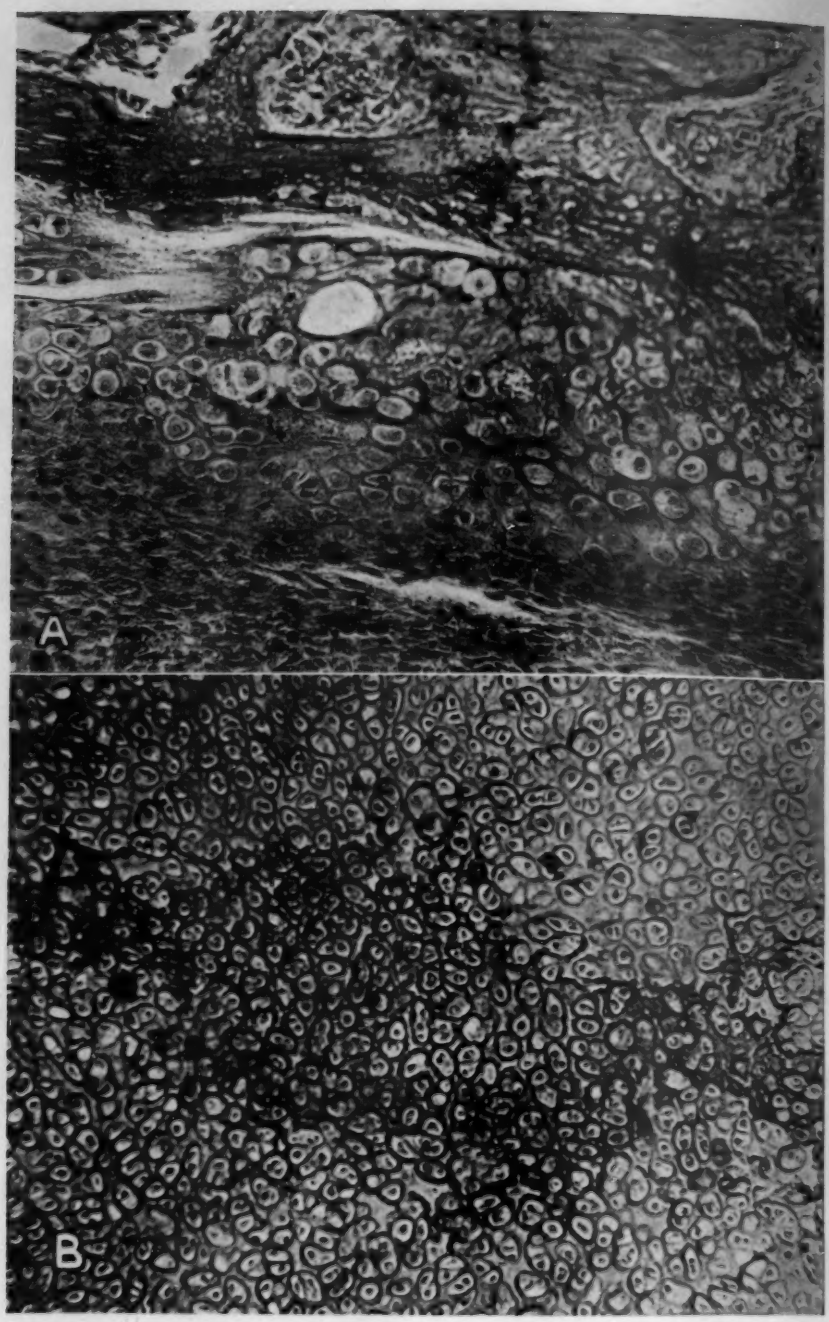

Fig. 5.—*A*, section from the shaft of the tibia of a male guinea pig which had received 1,040 rat units of estrogen in a watery solution over a period of two months; × 212.4. The weight of the animal increased from 180 to 435 Gm. At the top there is the bony shaft with marrow spaces. At the bottom there is the proliferating connective tissue of the periosteum. Between periosteum and bone there is an enclosure of cartilage which, below the marrow cavity at the right end of the picture, is seen penetrating into the bone. Some of the cartilage cells have undergone hypertrophy. Cystic enlargement of the cartilage in the middle of the picture is noted.

B, section through the cartilaginous part of a rib of a female guinea pig which had received 520 rat units of estrogen in a watery solution over a period of one month; magnification, same as in *A*. The weight of this animal increased from 175 to 265 Gm. Marked hyalinization of the intercartilaginous matrix of the rib is indicated by the dark-appearing material.

hyalinized fibrils developed in the intercellular stroma. The fibrocytes proliferated markedly and were quickly converted into precartilaginous cells. Finally, owing to the stimulation of growth processes in the periosteal tissue, a solution of the bony substance could take place. As far as the periosteal changes as well as those affecting the other constituents of the ribs were concerned, one had to deal with structural alterations which were identical with those in the corresponding elements of the tibia. As a consequence of these various processes, enlargement and thickening, especially of the cartilaginous parts, resulted, which in advanced conditions produced a slight rosary-like swelling of the chondro-osseous junction. The behavior of the bone marrow corresponded to the findings in the long bones.

Vertebral Column.—In the intervertebral disks the mesenchymal stroma underwent retrogressive changes, which were indicated by loosening and vacuolation of the ground substance. Subsequently marked thickening and hyalinization of the fibrils were noticeable. At later stages the sclerosed matrix, which contained bundles of thick collagenous fibers, became distinctly denser than ordinarily and increased in amount. Alterations of the cells went hand in hand with changes in the stroma. The spheroid fibrocartilage cells, which usually are scarce, began to proliferate by way of amitotic division. They increased not only in number but also in size. The cell nuclei were enlarged, and the cytoplasm was rounded off. Thus in some cases structures resulted which showed all kinds of transitions between ordinary connective tissue cells and cartilage cells and which finally could not be distinguished from cells characteristic of euhyaline cartilage; in other instances the hyperplasia led to the production of dense connective tissue. Within three to four weeks after the beginning of the administration of estrogen, pyknosis and karyolysis of the fibrocartilage cells could be observed frequently. Retrogressive changes were accentuated the more, the more the cells increased in size.

The effects noted on the vertebrae corresponded in all details to those seen in the long bones. As to the zones of endochondral ossification, degenerative processes were followed by hyalinization of the intercartilaginous chondromucoid matrix and its conversion into thick collagenous acidophilic masses, which became similar to osseous ground substance. Owing to predominance of ossification processes over new formation and differentiation of cartilage cells, the epiphysial line became narrowed at first. Subsequent growth, in particular of the columnar cartilage cells, as evidenced by nuclear divisions associated with cellular hypertrophy, led to temporary enlargement of the zones of ossification. Mitotic proliferation of the euhyaline cartilage cells covering the intervertebral joints was observed in cases in which nuclear division had occurred also in the epiphysial cartilage cells. Ultimately,

whole cell rows of proliferating cartilage degenerated and broke down. Such degenerated areas were quickly replaced by bone. Thus numerous thick wedgelike osseous plugs were found extending from the marrow throughout the whole epiphysis (fig. 2*A*). These plugs occasionally contained cartilage cells which were still recognizable and which were being converted into osteocytes.

After one to two months' treatment with estrogen these plugs became so large and numerous that the epiphysial line appeared almost ossified. The processes of apposition and solution which took place at the inner border of the periosteum and around the trabeculae of the bone marrow were comparable to those seen in the long bones; likewise, the changes in the bone marrow and its various elements were the same.

COMMENT

The action of estrogen on cartilage and bone in the immature guinea pig is shown as follows: At first, loosening and vacuolation of the intercartilaginous chondromucoid ground substance take place. This condition is associated with temporary suppression of the differentiation and growth of the resting and proliferating cartilage, wheras the ossification of the hypertrophic cartilage is accelerated. As a result of these changes, the zones of endochondral ossification become narrower. In the joints the layer of hypertrophic cartilage also diminishes in width and subsequently undergoes ossification. The bone marrow becomes fatty; an apposition of epithelioid osteoblasts along the trabeculae causes thickening of the bony spicules, which may also increase in number. In the subepiphysial layer, at the endosteum and in the periosteum intensified growth processes of the connective tissue lead to development of a fibrous tissue in which numerous collagenous fibers are sclerosed. The retrogressive changes which gradually progress in the cartilaginous matrix may also affect the cartilage cells themselves; karyolysis and karyorrhexis occur, and a loss of the normal structure of the epiphysial line results. Later, the growth processes of the euhyaline cartilage are resumed; hyperplasia and hypertrophy are increased above the normal level. Thus the zones of endochondral ossification and the cartilaginous covering of the joints appear widened. In the cartilage cells of the joint the hyperplastic and hypertrophic changes are distinctly more accentuated in the female than in the male guinea pigs. In the various cartilaginous tissues the sclerosed intercellular matrix increases in amount and becomes more and more similar to osseous ground substance. With increase in the production of sclerosed connective tissue, the ameboid movement of the osteoblastic epithelioid cells in the neighborhood of the trabeculae of the bone marrow and in the periosteal cambium layer is inhibited, and in the osseous structures solution processes predominate over the apposition of bone elements.

At still later stages, in the zones of endochondral ossification either the proliferating cartilage undergoes calcification, breakdown and replacement by bone or irregular circumscribed accelerated metaplastic conversion of columnar and hypertrophic cartilage cells into osteocytes leads to the formation of osseous plugs. This condition causes a second narrowing with partial ossification of the epiphysial lines and complete ossification of the cartilage cells of the pressure and transitional layers in the joints. On the other hand, the bony constituents of the marrow cavity assume again more normal appearances. The formation of cells resembling hemocytoblasts as well as of megakaryocytes is increased. In the periosteum, conversion of connective tissue cells into hypertrophic precartilage and ultimately into typical euhyaline cartilage cells is noticeable. These cartilage cells which are deposited along the bony compact layer of the shaft invade the osseous substance; they may undergo marked hypertrophy and also some degeneration.

The degree of the changes thus induced varies in different cases and is determined by the time during which the estrogen acts, the amount of the substance administered and the speed of resorption of this substance.

We must then conclude that the effect of estrogen on the skeletal tissues is a rather complex process. It cannot be defined as mere ossification of the epiphysial disks with subsequent cessation of growth in the lengthwise direction, as described by Tausk and de Fremery [5] in castrated dogs treated with an estrogen for twelve days, nor as hyperostotic osteoclerotic growth in the shaft and the marrow cavity of the long bones, changes which have been observed by Zondek [6] in fowls and by Gardner and Pfeiffer in mice [7a] and pigeons.[7b]

As far as the effect on the epiphysial line is concerned, a fairly general agreement seems to exist that estrogen produces narrowing and subsequent closure of this structure. Zondek found persistence of the epiphysial disks with simultaneous arrest of growth of the long bones, a condition which he identifies as pituitary dwarfism caused by inhibiting action of the estrogen on the pituitary gland. Only two investigations have come to our attention in which a histologic analysis of the epiphysial changes was made. One of them was carried out by Coryn,[8] who administered gonadotropic substances to castrated rabbits, and the other by Seeman [9] who, on the assumption that the narrowing of

5. Tausk, M., and de Fremery, P.: Acta brev. Neerl. **5**:19, 1935.

6. Zondek, B.: Lancet **2**:842, 1936.

7. Gardner, W. U., and Pfeiffer, C. A.: (*a*) Proc. Soc. Exper. Biol. & Med. **37**:678, 1938; (*b*) Endocrinology **23**:485, 1938.

8. Coryn, G.: Presse méd. **45**:1649, 1937.

9. Seeman, H.: Endokrinologie **18**:225, 1937.

the epiphysial line under the influence of estrogen was definitely proved, used this effect as the basis of a test for the presence of estrogen in the testicles of hogs. However, it may be stated that not only estrogen but also an extract of the anterior lobe of the pituitary gland may lead to narrowing of the epiphysial line[10] and that, as our experiments have shown, estrogen at certain stages may, on the contrary, cause widening of the zones of ossification.

As to new formation of bone, we have observed accelerated and intensified ossification in the preexisting zones of ossification and also in the cartilage of the joint. The same observation seems to apply also to the cartilage of the ribs, where the hyalinizing processes may perhaps be considered as precursors of ossification. But this interpretation will have to be verified by further experiments, which must be extended over longer periods.

However, with the exception of temporary apposition of bone around the trabeculae and likewise temporary increase in the number of the trabeculae in the epiphyses and diaphyses during the first four weeks of treatment, we have not observed any appreciable apposition of bone, either periosteally or endosteally, sufficient to cause thickening of the compact bone or narrowing and occlusion of the lumen of the marrow cavity. On the contrary, we have seen resorptive processes of considerable intensity acting on the cortex of the bones as well as on the trabeculae of the bone marrow after the first month of treatment.

In attempting to interpret the action of estrogen on the skeletal tissues we have (1) to distinguish between the effects of this substance on the various cellular constituents and those on the intercellular matrix and (2) to determine, if possible, which of the changes described are produced by the hormone itself, which may be due to a secondary hormonal influence and which may be induced by altered conditions in the affected tissues. It must be borne in mind that ossification even under normal circumstances is constantly controlled by both general and local factors and that a disturbance in either may alter the course of ossification.

Two different effects are exerted by estrogen on the cartilage cells of the epiphysial lines. At early stages and again after considerable amounts of the estrogen have been administered, definite suppression of growth occurs as far as size and number of cells are concerned. It is possible to explain this as a reversal of the effect of gonadectomy, which latter stimulates the growth of the cartilage by way of the pituitary gland.[1] Estrogen may inhibit the effect of the anterior lobe of the pituitary gland in a nonspecific way.

This explanation, however, cannot hold good for the second effect, observed in the intermediary stage, in which the epiphysial line is

10. Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **32**:1423, 1935.

widened and the number of the columnar cells is increased. At present we cannot harmonize these opposite effects with each other unless we attribute the last named to local conditions. The degenerative changes which take place within the matrix may perhaps exert a stimulating influence on neighboring cells, causing them to multiply and to assume the appearance of epithelioid cells. This condition persists up to the point when ossification advances from the marrow and prevents the cartilage cells from further proliferation; it is found also in the cartilage of the joints, where the advancing hyalinization and ossification of the matrix is accompanied by growth processes of the cartilage of the adjoining zones.

In the intercartilaginous matrix initial swelling and vacuolation are followed by marked hyalinization and subsequent ossification. In this connection it may be of interest to mention that hyalinization of the connective tissue and muscle tissue of the uterus and vagina of the mouse has been observed by Loeb, Suntzeff and Burns [11] following injections of large quantities of estrogen and that this process initiated foreign body reactions in the surrounding connective tissue. The question arises as to whether the hyalinization of the cartilaginous matrix, which initiates the metaplasia of the cartilage cells into osteocytes, is to be considered as the first stage of the process of ossification or whether it creates merely a local condition which in the case of the tissues possessing the ability to form bone is favorable to increased production of bone.

The rather widespread resorptive processes, which occur at the cortex with the cooperation of periosteal cells and about the trabeculae under the influence of connective tissue constituents of the bone marrow, are an expression of destructive action counterbalancing the bone building functions which these cellular elements exert under other conditions. We have observed a great number of giant cells, which dissolve the bony substance. The connective tissue may penetrate rather deeply into the cortex and here form small plugs surrounded by bone. In these areas as well as in others adjoining the bone from the periosteal side, cartilage is frequently found. We cannot as yet decide whether this intense ectopic cartilage formation is induced by hormonal factors or whether it is a consequence of local conditions, the nature of which needs further investigation. In this connection we may refer to the observations of Zawisch-Ossenitz,[12] who described the occurrence of islands of "basophilic substance" in the diaphysis of the long bones in various species of animals. She refers to the similarity which this basophilic substance exhibits to precartilage substance.

11. Loeb, L.; Suntzeff, V., and Burns, E. L.: Science **88**:432, 1938; Am. J. Cancer **35**:159, 1939.

12. Zawisch-Ossenitz, C.: Ztschr. f. mikr.-anat. Forsch. **10**:473, 1927.

SUMMARY

Estrogen administered to immature guinea pigs over periods of from three to sixty days causes retrogressive changes, increased hyalinization and ossification of the intercartilaginous ground substance in the epiphysial disks, ribs and vertebrae.

The cartilage cells respond with temporary cessation of growth and differentiation, followed by degenerative changes and subsequent proliferation and hypertrophy. The growth processes are accompanied by an accelerated and intensified breakdown of the growing cartilage and replacement by bone or by an accentuated direct metaplasia of columnar and hypertrophic cartilage cells into osteocytes. These processes may be considered as premature aging of the cartilage.

A transitory deposition of osteoid tissue around the preexisting trabeculae is followed by resorptive processes of the bony substance. A strong proliferation of the mesenchymal stroma of the bone marrow and the periosteum leads to the development of a sclerosed fibrous tissue. At present it is not certain that these effects are specific to certain hormones. One may also consider the possibility that in addition local factors play a role in the development of these processes.

THE ANITSCHKOW "MYOCYTE"

JOSEPH C. EHRLICH, M.D.
AND
BERNARD LAPAN, M.D.
NEW YORK

In 1913 Anitschkow [1] published a report of his experimental studies on the formation of granulation tissue in the myocardium. In this paper he discussed at some length a cell in the myocardium to which he gave the designation "myocyte." This cell was one of characteristic and constant appearance. It was distinguished by the presence of a peculiar longitudinal bar or rod within the nucleus, composed presumably of condensed chromatin substance; this bar had a serrate edge and was separated from the nuclear membrane by a clear zone on all sides (fig. 1*A*).

Anitschkow's attention was drawn to this cell by its occurrence in granulation tissue which had been experimentally produced by introducing foreign bodies into the myocardium of rabbits. This "myocyte" had previously been observed and superficially described, particularly by Von Oppel [2] in 1901 and Saltykow [3] in 1905. Both these investigators and also a number of subsequent observers, including chiefly Karsner and Dwyer,[4] Jacki,[5] Wätjen,[6] Hesse and Hesse [7] and Semsroth and Pool,[8] had their attention drawn to the "myocyte" in the course of studies which were concerned with the reaction of the myocardium to traumatic injuries, foreign bodies, inflammation and infarction produced by ligation of coronary arteries and also with the question of regeneration of the myocardium following its destruction from one cause or another.

From the Laboratories of Lebanon Hospital and Mount Sinai Hospital.

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In 1929 there appeared a paper by Wenezianowa-Grusdkowa [9] devoted specifically to the Anitschkow "myocyte," in which the question of the origin of this cell was considered from the standpoint of modern histogenic concepts and in which a more thorough attempt was made to discover the nature of the cell by histologic studies and by intravital injections of dye.

In 1938 Richard H. Jaffé,[10] in his monograph on the reticuloendothelial system, presented a rather complete, though short, discussion of the Anitschkow "myocyte."

In the present study the question of the origin and significance of the Anitschkow "myocyte" is taken up anew on the basis of embryologic and comparative anatomic observations.

MATERIAL AND METHODS

For the purpose of this study, numerous hearts were examined in the following general groupings: (1) human age series, (2) human embryo series, (3) chick embryo series and (4) general animal series. The animal series comprised hearts from over twenty-five different species and included almost the entire subphylum of vertebrates. The hearts were examined chiefly in thin paraffin sections stained with hematoxylin and eosin and occasionally with other stains, such as Van Gieson's and the silver impregnation stain. In addition, representative sections were made of fresh unfixed material prepared by the frozen section method and stained with hematoxylin and eosin. Also, frozen sections of fixed material were examined. Fixation was always by formaldehyde.

Because of the distinctive intranuclear formation present in the cells under consideration and because of the occurrence of these cells in a rhythmically pulsating organ, namely, the heart, the possibility of artefactual influences determining their appearance in sections was taken into consideration. However, examination of fresh unfixed material by the frozen section method and examination of thin blocks of fresh unfixed tissue prepared without freezing and examined by the Terry method (utilizing polychrome methylene blue) disclosed intranuclear formations identical with those observed in fixed paraffin sections. Semsroth and Pool [8] also felt that the distinctive nuclear appearance was not an artefact, chiefly because other nuclei in the vicinity were of normal appearance.

GENERAL DESCRIPTION

This investigation has been concerned with a cell (fig. 1*A*) which shows a characteristic appearance under a high power objective. This appearance is very striking, and in the vast majority of instances it is clearly distinguishable from that of all other nuclei occurring not only within the heart but, so far as our experience goes, in other tissues of the body as well. In the normal heart during the postnatal period, in both man and animals, this cell appears practically free from distinguish-

9. Wenezianowa-Grusdkowa, M. S.: Frankfurt. Ztschr. f. Path. **37**:538, 1929.

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able cytoplasm. Occasionally a small perinuclear zone of cytoplasm may be observed at either pole of the cell (fig. 1 *C*). Such cytoplasmic tabs, when present, are clear, homogeneous and slightly basophilic and taper out rapidly into thin strands at each end of the cell, which dis-

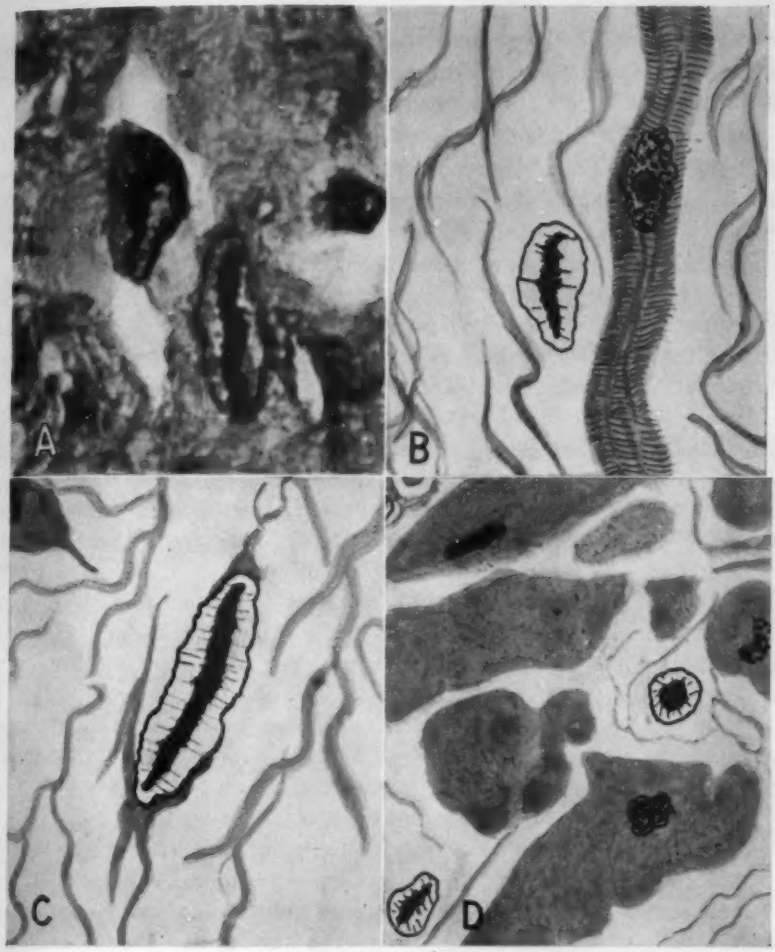

Fig. 1.—*A*, human heart valve at the age of 8 years; photomicrograph; × 1,800. Two "myocytes" are seen sectioned longitudinally. *B*, human heart at the age of 6 months; camera lucida drawing; × 1,800. A "myocyte" in longitudinal section adjoins a muscle fiber. *C*, adult frog heart; camera lucida drawing; × 3,000. A "myocyte" is seen in longitudinal section, with tabs of cytoplasm visible at both poles. A portion of a fibroblast is seen above to the left. *D*, human heart at the age of 6 months; camera lucida drawing; × 1,200. At the right of center is a "myocyte" in cross section. A "myocyte" in longitudinal section is seen at the lower left. Note the three muscle nuclei within muscle fibers.

appear into the ground substance. In the experiments of Wenezianowa-Grusdkowa,[9] the cytoplasm of the "myocytes" was clearly indicated in those instances in which these cells stored intravital dye.

The special morphologic characteristics of this cell are concerned with the nucleus. The appearance presented is almost constant in any given plane regardless of the part of the heart in which the cell is encountered or of the prenatal or postnatal age period in which the heart is examined. The nucleus as seen in longitudinal section appears as a rather uniformly elliptic structure with a sharply outlined, thin uniform membrane, which stains darkly with hematoxylin. The intranuclear space appears empty or vacuolated, and within this clear zone, occupying the central portion of the intranuclear space, there is a conspicuous rod-shaped body. This body varies somewhat in thickness, being usually tapered toward the poles, and its outline is slightly serrated. This body always takes a deep nuclear stain. The general configuration of this rod is such that it occupies almost the entire long axis of the nucleus, reaching at the tapered poles almost to the nuclear membrane. At its widest point, which is usually near the center, the width of this rod-shaped structure is approximately one-third that of the nucleus, and because of its central location there remains on each side of it a clear zone which is equal also to approximately one-third the width of the nucleus. Radiating from the serrated edges of the rodlike body, usually at right angles to its long axis, are extremely fine fibrillar structures which extend directly from the side of the rod-shaped body toward and in many cases up to the nuclear membrane. These threadlike structures cannot be observed emanating from all the toothlike projections in this intranuclear structure but are quite evenly distributed at short intervals. Within the nucleus no other constant chromatin elements or nucleoli can be observed, and those parts of the intranuclear space which are not occupied by this intranuclear body or by its radiating strands appear entirely clear.

In cross section the nucleus appears as a small circle in the center of which is a dark circular body with a serrated edge from which strands radiate toward and to the nuclear membrane. The appearance of these strands indicates that they radiate from the surface of the intranuclear body in all planes (fig. 1 *D*). We have occasionally observed a lighter staining zone in the center of the intranuclear body.

Occasional specimens, chiefly the 18 day embryo chick heart, presented clusters of these cells with abundant cytoplasm (fig. 2 *A*). Such cells were stellate or polygonal in shape, with a rather abundant amount of clear homogeneous basophilic cytoplasm. The stellate projections of cytoplasm tapered off into fine fibrillar strands and were lost in the surrounding ground substance.

Similar cytoplasmic increases have been observed by us in human hearts—for example, in rheumatic fever. We have on rare occasions observed nuclei of suggestively similar structure in other tissues. In an instance of lipoid pneumonia in a child, with metaplastic changes in the tracheobronchial mucosa, the metaplastic tissue presented one epithelial cell of similar structure. Occasionally the nuclei of cardiac muscle fibers when there are pronounced longitudinal folds in the nuclear membrane may bear a superficial resemblance to the cell described. This artificial resemblance is quickly clarified by study of a cross section of the nuclear membrane. We have not made any extensive examinations of other tissues of the body from the standpoint of whether or not similar cells may be present outside of the heart, although in our general experience with human tissues, such cells have not been encountered. Nevertheless it is possible that further studies might reveal similar or identical nuclei outside the heart.

GENERAL DISTRIBUTION

In an average normal human heart, for example, during the third decade of life "myocytes" may be found distributed throughout the entire musculature. Within the ventricles, in the intraventricular septum and in the papillary muscles, extending all the way up to the auriculoventricular ring, these cells are frequently encountered with the high power objective. Also in the auricular musculature and in the substance of the mitral, tricuspid, aortic and pulmonary valves these cells are found. All four valve rings contain numerous "myocytes."

Within the myocardium, they may be found most easily by searching in the interfascicular planes. These planes, in which the larger blood vessels run before their break-up into the capillaries which ramify within the cardiac muscle fasciculi, are composed of loosely arranged collagen bundles and argyrophilic fibers. By searching along such planes, occasional "myocytes" will be encountered, in some areas more than in others, along with the usual cellular elements present in this tissue, namely, fibroblasts, occasional macrophages, a rare mast cell and the nuclei of arterioles, venules and lymphatics, with their perivascular cells. The nucleus of the "myocyte" in such an area is so arranged that its long axis is usually parallel to the collagen fibers running in these planes. The "myocytes," however, are not confined to the interfascicular planes but may be found also within the muscle fasciculi. In such instances they lie in close proximity to individual cardiac muscle fibers (fig. 1 *A* and *B*). In many instances, they appear to lie within these fibers, although examination of the cross section reveals this to be the result of superposition or infolding. For the most part, "myocytes" lie external to the sarcolemma of the cardiac muscle fibers.

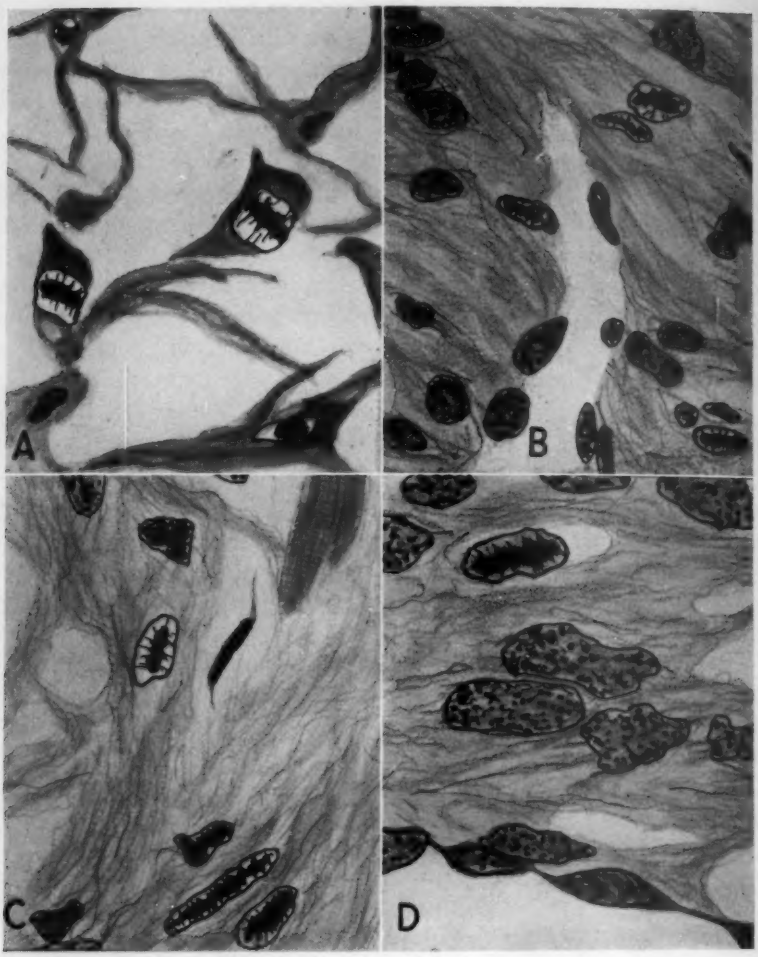

Fig. 2.—*A*, chick embryo heart at 18 days; camera lucida drawing; × 1,200. An area in the valve cushion is shown in which "myocytes" may be seen with distinct cytoplasm containing zones of basophilia and tapering off into the surrounding ground substance. *B*, rabbit embryo heart at about 20 days; camera lucida drawing; × 950. An endocardial indipping in the ventricular wall is seen. To the left of center is a "myocyte" closely applied to an endothelial cell nucleus. Striations are not yet visible in the cardiac muscle. Numerous small and large muscle nuclei are present and several "myocytes." *C*, adult fish (carp) heart; camera lucida drawing; × 950. Distinct "myocytes" are present. A muscle fiber is seen above to the right. *D*, human embryo heart at 1 month; camera lucida drawing; × 1,400. A "myocyte" is seen a short distance from the endothelium lining the primitive ventricle. Large muscle nuclei and immature muscle fibers are present. Muscle striations are not yet visible.

In the cardiac valve these cells are occasionally encountered in the fibrous body of the valve along with other common cells such as fibroblasts and occasional macrophages and round cells.

Many "myocytes" may be found beneath the endocardium not only in the heart valves but also elsewhere in the heart, where they may be traced following the complex indippings of the endocardium among the muscular trabeculae of the ventricles (fig. *2B*).

In the human embryo series and in a series of 20 chick embryos, aged 1 to 21 days, "myocytes" were found with ease. In the chick embryo heart, they were observed first on the twelfth day, and the youngest human embryo heart in which these cells were observed was about 1 month old. In both these embryos the earliest "myocytes" were found immediately beneath the endothelium of the ventricle and appeared to be related to the distribution of endocardium, inasmuch as they were found first not only beneath the endothelium in the ventricles but also in those endocardial aggregations which constitute the valve cushions. For example, in early embryos, with valve cushions in the earliest stage of their formation, the main substance of these cushions contained relatively large numbers of "myocytes." Indeed, the substance of the valve cushions and later the valves themselves contained large numbers of "myocytes" throughout the entire stage of embryonal development (fig. *2A*). Within the myocardium the appearance of these cells and their penetration throughout the muscle seem to follow the complicated endocardial indippings which permeate the entire myocardium during the earliest stages of the development of the heart, and it appears probable to us that this cell reaches all parts of the myocardium during this stage of development (fig. *2B* and *D*). By the fifth month, in the human embryo, large numbers of "myocytes" may be found throughout the myocardium, although their numbers appear to be relatively diminished during the later stages of embryonal life.

As regards the general animal series, "myocytes" were found without difficulty in all hearts examined.[11] (See fig. 1*C* for the frog, fig. *2B* for the rabbit and fig. *2C* for the fish.)

Examination of human hearts of the first to the eighth decade of life showed a rather constant quantitative and qualitative distribution of "myocytes."

COMMENT

The foregoing anatomic observations indicate that the "myocyte" is a cell of distinctive morphology, whose development and topographic relationships suggest that it constitutes part of the supporting framework of the myocardium and valves.

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11. Hearts were studied in the following animals: fish (carp); amphibians (frog); reptiles (boa, turtle, iguana, lizzard); birds (chicken, crane, ibis, pelican, owl, parrot, hawk), and mammals (armadillo, polar bear, tahr, sheep, sea lion, rabbit, guinea pig, cat, monkey [mandrill, capuchin, marmoset, macaque]).

This conclusion is based on the location of these cells in the interfascicular planes, in the fibrous core of the cardiac valves and within the muscle fasciculae between the individual cardiac muscle fibers, i. e., external to the sarcolemma fibers. Furthermore, the polygonal or stellate form, tapering out into fine cytoplasmic fibrillae (when cytoplasm is observed), conforms to the general morphology of interstitial cellular elements. They do not appear, from our studies, to bear any relationship to specially differentiated myocardial fibers (Purkinje system). Because they are distributed mainly in the interfascicular planes, they appear to be present rather frequently in the vicinity of the smaller divisions of the coronary arteries which travel in these planes before ramifying as capillaries within the cardiac muscle fasciculae.

From an embryogenic standpoint, our observations indicate that "myocytes" arise predominantly as perivascular cells, inasmuch as they appear first just external to the endothelial lining of the primitive heart. It appears further that the "myocytes" permeate the entire myocardium in that stage of embryonal development of the heart in which the myocardium consists of a loose, trabecular spongework of muscle fibers, clothed by endocardial extensions and indippings. Further evidence of the derivation of these cells from the subendothelial layer of the endocardium is offered by the large number of "myocytes" present within the endocardial cushions, which represent the earlier stages in the formation of the cardiac valves.

Previous conceptions of the derivation of the "myocyte" from degenerated muscle cells were undoubtedly the result of histologic methods which were not adequate for the separate distinction of "myocyte" nuclei closely applied to muscle fibers, as well as of the fact that these cells were studied incidentally to observations carried out on areas of degenerated myocardial fibers. It is evident that had the hearts of embryos and the newborn of both animals and man been studied, in both of which "myocytes" are numerous and easy to find, the question of the origin of these cells from degenerating muscle would never have been even considered. Later observers like Wätjen [6] and Semsroth and Pool,[8] in fact, gave up the idea of a myocardial orgin because the former found typical "myocytes" in the epicardium in a case of rheumatic myocarditis, and the latter, at the base of vegetations on the mitral valves, in both of which areas cardiac muscle tissue is not present. Anitschkow's [1] discussion and illustration of intermediate stages in the transformation of myocardial fibers through degeneration into "myocytes" must therefore be revised on the basis of the observations reported by later investigators and those which we have recorded here.

Nevertheless, the observations of Saltykow,[3] Von Oppel,[2] Anitschkow [1] and others relating to the prominent appearance of "myocytes" in processes reactive to injury, inflammation or foreign body irritation

in the myocardium are correct. We also have observed proliferative and reactive changes in the "myocytes" in a number of other cardiac pathologic states. These include sepsis, meningococcic bacteremia, scarlet fever, periarteritis nodosa and subacute bacterial endocarditis. Recently our attention has been drawn to a peculiar focal granuloma in the myocardium by Dr. Paul Klemperer, in which again the "myocytes" showed some proliferation. Anitschkow [1] and others have already observed that not only are "myocytes" present in the damaged region in significant numbers but their cytoplasm is prominent and distinctly basophilic in tone. These observations have been confirmed by us and appear to signify further a dedifferentiation to the embryonal stage, since it is precisely this prominence of the cytoplasm and its basophilic tone which characterize these cells in certain stages of their embryonal development, as described in a previous section of this paper. The observations of the authors quoted regarding the activity of the "myocytes" in reparative processes require much further study, especially since the distinctive appearance of "myocytes" makes it possible to trace with ease much of the morphologic alteration which they may undergo in disease processes. Jacki [5] and Wätjen [6] noted the resemblance of the nuclei in certain cells which compose the Aschoff body to "myocytes," and Jaffé [10] also referred to this resemblance. One of us (J. C. E.) has made this observation also, and a separate paper devoted to this question is now in preparation. The resemblance of the "myocytes" to certain cells constituting the Aschoff body may well have played a part in the concept which has been prominent for some time that the Aschoff body is a derivative of necrobiotic cardiac muscle fibers.

Of the various sources which have been proposed theoretically for the origin of the "myocytes," namely, degenerating muscle, vascular endothelium, fibroblasts and histiocytes, only the last appears to us to be tenable on the basis of our own observations. Wenezianowa-Grusdkowa [9] ascribed a reticuloendothelial origin to these cells because of their ability to store intravital dyes. (Anitschkow [1] also mentioned the ability of these cells to function as phagocytes.) Jaffé [10] did not accept Wenezianowa-Grusdkowa's evidence of storage capacity as a proof that this cell is not derived from muscle, contending that muscle may also store intravital dye under certain conditions. Inasmuch, however, as the storage of dye in muscle requires tremendous overloading with dye, which was apparently not the case in Wenezianowa-Grusdkowa's experiments, we are inclined to accept the existing evidence that the "myocyte" may assume an active phagocytic function.

However, we do not agree with Wenezianowa-Grusdkowa's suggestion that the "myocytes" are derived from free or circulating elements of the reticuloendothelial system (polyblasts). Our observations indicate rather that these cells belong to the fixed elements of the reticulo-

endothelial system in the myocardial framework, as suggested by Jaffé, being derived originally from subendothelial or perivascular fixed cells. The active participation of the "myocyte" in the aforementioned reactive states of the myocardium, and no doubt in many others not yet investigated, is entirely consistent with the role played by the reticuloendothelial system in reparative processes.

It is evident from the foregoing discussion that fundamental objections exist to the continued use of the term "myocyte" for these cells. We feel that the characteristic appearance of this cell and its essential nature can be better expressed by the term "myocardial reticulocyte."

SUMMARY

The Anitschkow "myocyte" is a normal constituent of the human and vertebrate heart during the embryonal and postembryonal stages of development. This cell is part of the supporting tissue of the heart and belongs to the fixed elements of the reticuloendothelial system. It plays a definite role in inflammatory and other defense reactions. The name "myocardial reticulocyte" is suggested as a more accurate designation for this cell.

CHANGES IN THE PROSTATE CAUSED BY HIGH FREQUENCY CURRENT

LEO A. MASLOW, M.D.
CHICAGO
AND
EUGEN MARTOS, M.D.
BUDAPEST, HUNGARY

The introduction and popularization of transurethral prostatic resection created a diagnostic problem in pathology which requires clarification particularly for the clinical urologist and those unfamiliar with pathologic diagnosis. The difficulty arises from the fact that the current produces a distortion of tissues which gives a confusing picture and makes the pathologic interpretation difficult.

The changes produced by transurethral resection are of two kinds: The first kind includes those found in the tissues removed with the resectoscope; these changes are produced at the moment of penetration of the tissue by the current. To the second kind belong those changes which are a reaction of the organism and which appear in the prostatic tissue remaining in the body, after a few hours or longer. The secondary changes are very important from the clinical point of view. whereas the primary effect of the procedure on the tissues is interesting rather from the pathologic point of view.

The effect of high heat and various currents on other tissues has been examined by many authors. But we found only two publications dealing specifically with our subject. Caulk and Harris [1] published their microscopic observations in connection with operations on dogs and 2 human subjects. They mentioned in general the superficial necrosis and the shrinking of cells and nuclei in the material examined immediately after operation, but they did not go into details. They found also in the prostate removed a variable time after resection secondary necrobiotic changes. Smith and Stockwell [2] examined the effect of electrodehydration on prostatic resection. In 1 case they examined the prostate twelve hours after resection and in their microscopic examination found only the necrosis of the superficial tissue

From St. Istvan Hospital, Budapest, Hungary, under the direction of Prof. Odon Zalka.

1. Caulk, J. R., and Harris, W.: J. Urol. **32**:473, 1933.
2. Smith, C. K., and Stockwell, L.: J. Urol. **34**:31, 1935.

and hemorrhagic infiltration significant. They did not find the secondary changes described by Caulk and Harris.

Our work consisted in the examination of pieces of prostatic tissue removed from 10 human subjects by transurethral resection and 40 prostates removed either from cadavers or from patients by prostatectomy, to which the usual resection procedure had been applied. Tube type and spark gap type machines were employed. The strength of current used was 4 to 10 amperes; the duration of the application of the current varied from two to eight seconds.

We found in the microscopic picture of the tissues obtained by transurethral resection conspicuous and characteristic features, the most confusing of which is the elongation of cells and nuclei to be described later. If this occurs in the epithelial cells it produces fantastic, distorted glands, which make a correct diagnosis difficult.

MICROSCOPIC OBSERVATIONS

Since we found only small quantitative differences between the various cases of transurethral resection, we summarize our observations. We distinguish in general three layers in the microscopic picture. The most superficial forms a very thin, brownish yellow, torn, charred edge. Below this is a vacuolated, spongy layer, which is already well known in cases of electric injuries of other nature and damage of tissues caused by heat. This corresponds to the picture called *Wabenhof* or *Hitzewaben* by the German authors. In general, this layer is also thin, but sometimes it can be followed between the loose connective tissue down to the deeper layers. The septums which separate the vacuoles are homogeneous, and the original structure cannot be recognized in them, only one or two nuclei, which stain very lightly. This layer stains with hematoxylin diffusely light blue (fig. 1*A*).

The first and second layers are thin. The third layer is much more conspicuous and extensive, sometimes comprising the remaining part of the prostate, if the removed particles are small. In this layer the original tissue structure becomes indistinct, i. e., more or less homogeneous, especially in the firmer parts. Nevertheless it can be recognized. The fibers of the connective tissue become confluent, forming bundles which stain bluish with hematoxylin, and they lie close to the neighboring smooth muscle tissue cells. The latter also are confluent, forming hyalin-like islands between the light blue fibers of connective tissue, and they stain well with eosin. In the nuclei there can be observed also a vacuolated swelling. The walls of the small vessels also stain homogeneously light blue and form a ring lined with endo-

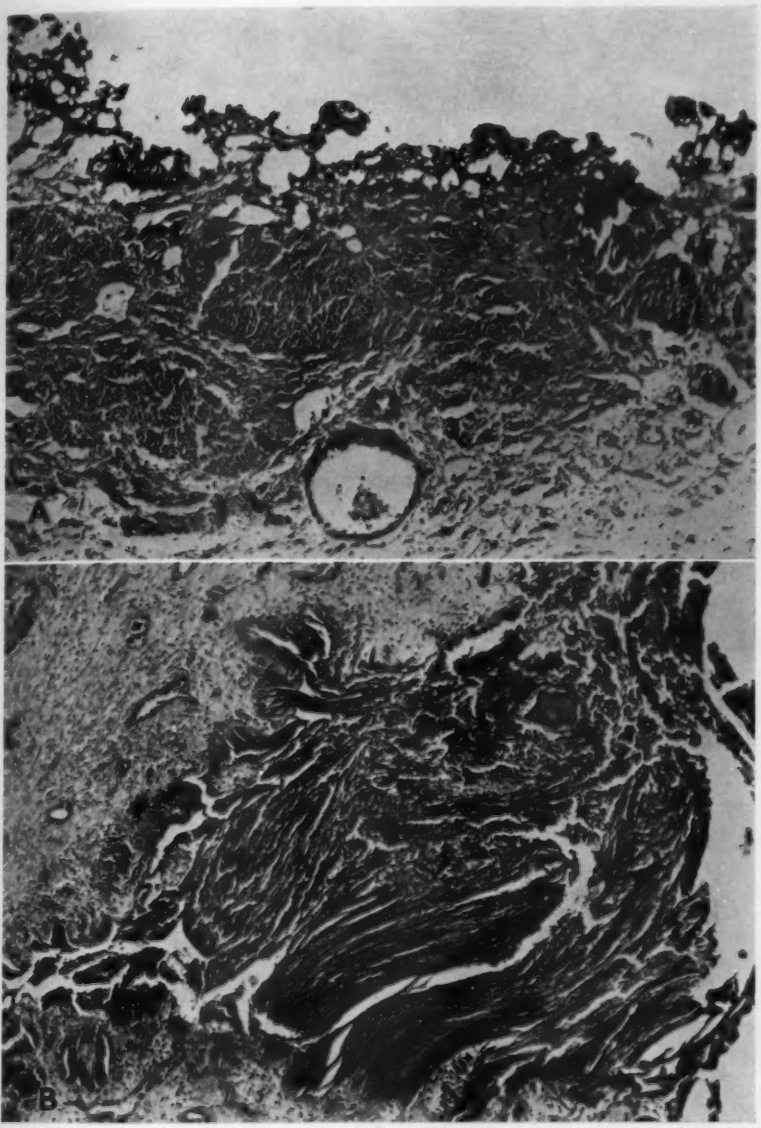

Fig. 1.—*A* shows the three layers: (*a*) the charred edge, (*b*) the spongy layer and (*c*) the coagulation layer. *B* shows the considerable elongation of the epithelial cells in the superficial layer.

thelium. In this layer the nuclei of the firmer fibrous connective tissue are deformed and shrunk, or they appear as either wavy or straight threadlike formations and are in general hypochromatic. The nuclei in the loose connective tissue around the vessels and glands are less shrunk and hyperchromatic.

In this layer the epithelium of the glands shows significant changes, the essential one being the considerable elongation of the epithelial cells and their nuclei (fig. 1*B*). The cells may become five or six times their original height. The elongation of the epithelial cells may reach such an extent that the glandular lumens can hardly be recognized in many places. In this way a picture may be seen which reminds one of solid islands. The protoplasm stains lightly. The place of the nucleus is sometimes taken by an elongated, threadlike formation. The staining of the elongated nuclei is generally intensive, and in certain parts it shows segmentation. The nuclei are generally parallel and are mostly straight or show one or two wavelike curvatures, the whole group of elongated nuclei showing a hair bundle–like picture (fig. 2*A*). Such alveoli, which show elongation of their nuclei and are in consequence distorted, occur not only in the connective tissue which becomes homogeneous but sometimes also in the deeper layers of connective tissue which do not show any artificial changes.

We performed the usual resection procedure on 40 prostates, removed either surgically or from cadavers, in order to examine whether the changes described are those of vital reaction or those merely of a passive physical nature. The prostates were preserved in a refrigerator, and the current was applied one half to six days after death.

We found on histologic examination changes similar to those found in the tissues removed from the human subject by transurethral resection, the differences being only quantitative (fig. 2*B*). The parts affected are essentially smaller, being only one-half or one-third as wide, and the effect of the current on the individual tissue elements is also considerably smaller. The elongation of the nuclei is much less, occurring only on the side of the gland which is close to the surface and showing a gradual decline toward the parts farther from the treated area. In such glands the nuclei which are not elongated show only a hyperchromatic character. When the resections have been done with different strengths of currents, there is no proportion between the severity of the changes and the intensity of the current. The time elapsing between death or operative removal of the gland and application of current seemed to play no role in the degree of changes found microscopically.

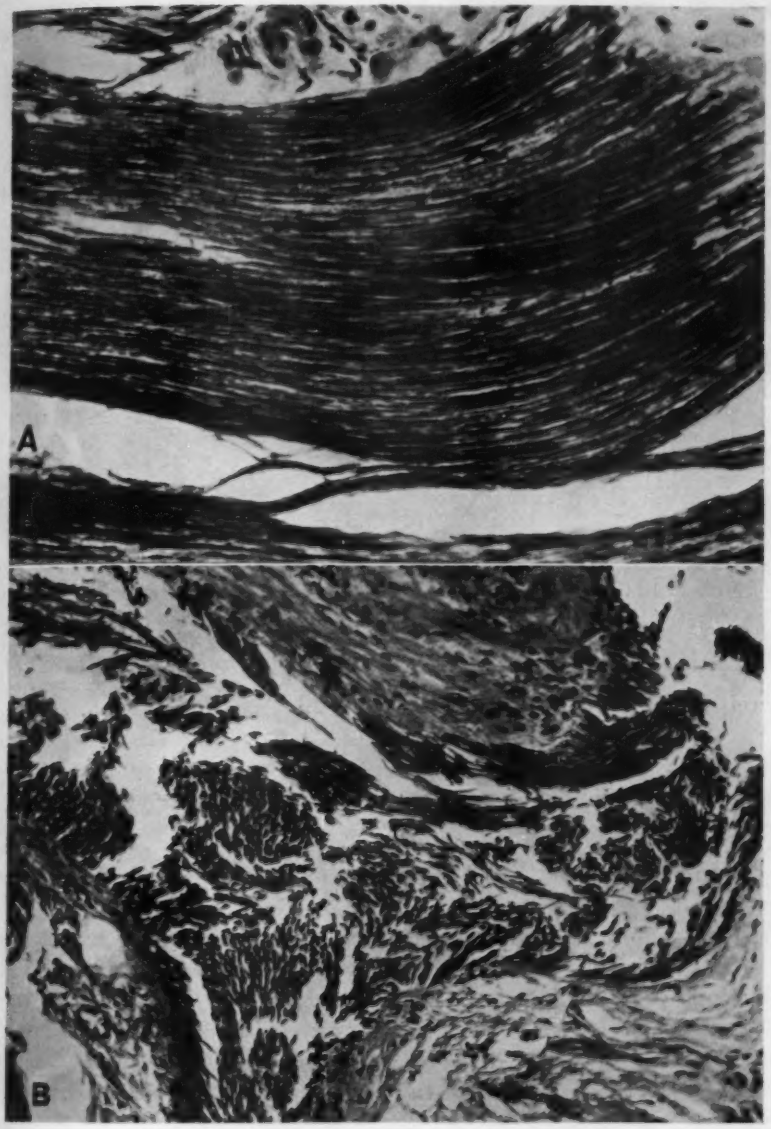

Fig. 2.—*A* shows the hair bundle-like picture presented by the elongated nuclei; *B* the nuclear elongation in a cadaver prostate five days after death.

COMMENT

Many authors have found changes in other organs and tissues caused by other agents similar to those found in transurethral prostatic resection. Especially conspicuous is the similarity of the characteristic elongation of the glandular epithelial cells found in the bronchial passages and in the gastrointestinal tract caused by heat (e. g., burning, inhalation of hot air, touching with red hot metal). As to the mechanism of the origin of the changes, opinions of the various authors differ. Some have stated that the effects of the current are due to heat, while others have found them to be the result of a specific electromechanic action. Pietrusky [3] and Schridde [4] stated that the electrolytic factor also plays a role. Many authors have found quite similar cell deformities due only to heat. In using electric current one cannot exclude its electrolytic and electrodynamic role, but we find Joul's heat effect the most important factor.

As to the question of whether or not the elongation of nuclei is of vital character, the result of our experiments on prostates removed from cadavers 5 to 6 days old convinces us that it is merely a physical phenomenon. The irregularity of the changes found in these prostates at varying periods after death and the quantitative differences of changes in the operative and cadaver material show that the degree of the effect of the resection procedure depends very much on the moisture content of the tissue. An observation of ours made on a prostate five days after removal speaks in favor of this supposition. This prostate was preserved in a closed bottle for five days, and we made the resection on the side which was in contact with the wall of the glass. In consequence of this, the loss of moisture was minimal. In this case we found a greater amount of nuclear elongation than in some specimens only 1 or 2 days old.

SUMMARY

Tissue particles obtained by transurethral prostatic resection show considerable distortion. The changes consist of three layers of different structure: a charred thin superficial layer, a spongy coagulative layer of varying width and a wider layer which becomes homogeneous in varying degrees.

The alveolar epithelium becomes much elongated, threadlike and packed in close bundles. Knowledge of the nuclear elongation is important because it may lead to diagnostic mistakes. We were able to produce similar but smaller changes in cadaver prostates five to six days after death. The degree of the changes produced seems to be independent of the strength of the current but seems to be dependent on the moisture content of the tissue.

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4. Schridde, H.: Virchows Arch. f. path. Anat. **252**:774, 1924.

MEDIAL DEGENERATION OF THE AORTA

A STUDY OF TWO HUNDRED AND TEN ROUTINE AUTOPSY SPECIMENS BY A SERIAL BLOCK METHOD

ANTONIO ROTTINO, M.D.

Pathologist of St. Vincent's Hospital and Instructor in the Department of Pathology of New York University Medical College

NEW YORK

It is proposed in this paper to present a survey of medial degeneration of the aorta as encountered in routine autopsy specimens. Weise [1] alone attempted a similar study. Others who described medial degeneration in the intact aorta studied selected [2] or isolated specimens.[3] The majority of workers write of medial degeneration in spontaneously ruptured aortas.[4] It must be mentioned that Moritz [5] also investigated the intact aorta, but his purpose was essentially to note the distribution of chromatotropic substances in the media.

MATERIAL

The material used in this study consists of 210 aortas from persons in all decades of life (table), who died from a variety of conditions. No attempt was made to select specimens, in order to insure as varied a group of diseases as possible. The large number precludes enumeration. However, persons from whom the specimens came died of such conditions as carcinoma, acute infections, heart disease of various types, primary blood dyscrasia (leukemia, pernicious anemia, agranulocytosis, aplastic anemia and others), cirrhosis of the liver, bleeding and perforated ulcers of the stomach, diabetes, trauma, gas poisoning, postpartum hemorrhage, Addison's disease, acute hemorrhagic pancreatitis and so on.

METHOD OF PREPARATION

The entire ascending aorta and arch were cut into serial transverse blocks, six to ten in number, 3 to 5 mm. in length, each block including the entire circumference of the vessel. In addition, longitudinal strips were selected from the thoracic aorta, two to four in number. From each block two sections were prepared, of which one was prepared with the hematoxylin-eosin stain and the other with Weigert's elastic tissue stain. In 150 cases a map of the aorta was made, on which was indicated the level of each section studied. This was done

1. Weise, W.: Beitr. z. path. Anat. u. z. allg. Path. **93**:238, 1934.

2. Cellina, M.: Virchows Arch. f. path. Anat. **280**:65, 1931.

3. Rottino, A.: Arch. Path. **27**:320, 1939. Harrison, F. F.: ibid. **27**:742, 1939.

4. (*a*) Gsell, O.: Vircows Arch. f. path. Anat. **270**:1, 1928. (*b*) Erdheim, J.: ibid. **273**:454, 1929; **276**:187, 1930.

5. Moritz, A. R.: Am. J. Path. **8**:717, 1932.

to make possible an accurate survey of the distribution of the lesion, especially as to its presence or absence in the descending portion of the vessel.

OBSERVATIONS

Degenerative changes beneath atherosclerotic plaques were not considered in this study. Despite this limitation, lesions were found in 95 aortas. The most common lesion, present in 92 aortas, was similar to one described in a previous communication [6] as simple muscle loss. It was characterized by loss of muscle cells from focal areas of media. Though the continuity of the elastic lamellae in these areas remained intact, the lamellae became crowded so as to narrow the interlamellar spaces (fig. 1 *A* and *B*). Anuclear muscle cell bodies such as those described by Gsell [4a] were not recognized. In 5 of the 92 aortas there were additional areas in which the elastic elements were disintegrated or in the process of disintegration, leaving defects which were occupied chiefly by a basophilic mucoid fluid (fig. 2 *A*) and in 1 specimen by a loose fibrillar connective tissue (fig. 2 *B*). In another aorta—the ninety-third—there were numerous irregular narrow foci in which elastic lamellae were absent while muscle cells remained (fig. 3 *A* and *B*). The latter were conspicuous in that their longitudinal axes lay perpendicular or oblique to those in adjacent normal areas. Cystic spaces filled with mucoid fluid were also present in this aorta, but nowhere were areas of simple muscle loss found. In the final 2 specimens the sole change consisted of cystic areas filled with mucoid fluid.

The lesions were distributed principally in the ascending aorta and arch. In 5 aortas they were found, in addition, in the descending thoracic portion and once only in the abdominal portion. Within the media the lesion was chiefly located in the middle, not infrequently in the inner and rarely in the outer third.

Based on the number and size of the lesions, the severity of the process was graded as 1 plus (50 cases), 2 plus (15 cases), 3 plus (6 cases), and 4 plus (24 cases). The 1 plus lesion was insignificant, one to three small areas of involvement being found in the entire aorta. In the 4 plus group as many as two to six lesions were found in every section. Further, they were large, involving as much as one quarter to one half of the thickness of the media. The lesions of 2 plus and 3 plus grades occupied intermediate positions of severity. In none of the cases was a cellular reaction present in or adjacent to the lesions described. No relationship was encountered between the presence of the lesion and changes either in the vasa vasorum or in the adventitia.

ANALYSIS OF OBSERVATIONS

In an earlier published report [6] on medial degeneration as encountered in the ruptured aorta, advanced age, hypertension and some

6. Rottino, A.: Arch. Path. **28**:1, 1939.

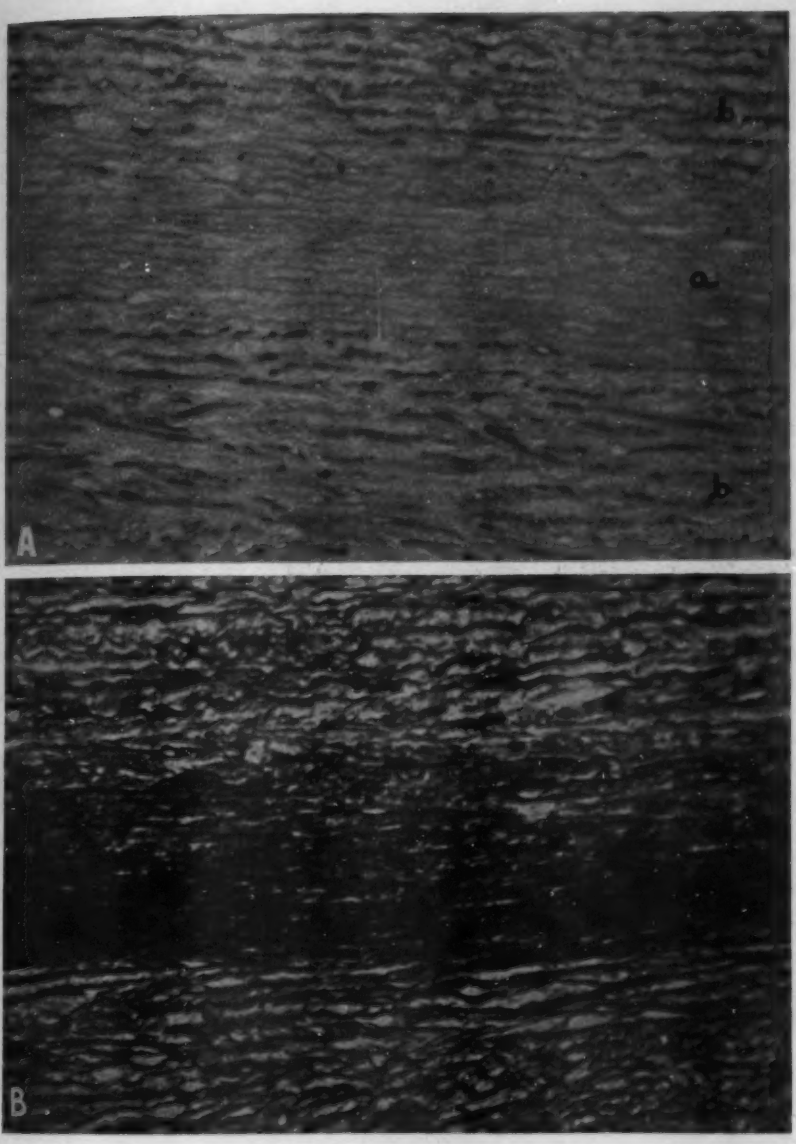

Fig 1.—*A*, hematoxylin-eosin stain showing, under low power magnification, (*a*) an area of simple loss of muscle and (*b*) normal media. *B*, Weigert's elastic tissue stain showing, under low power magnification, crowding of elastic laminas and narrowing of interlamellar spaces in an area of simple loss of muscle. In adjacent areas the lamellae are properly spaced.

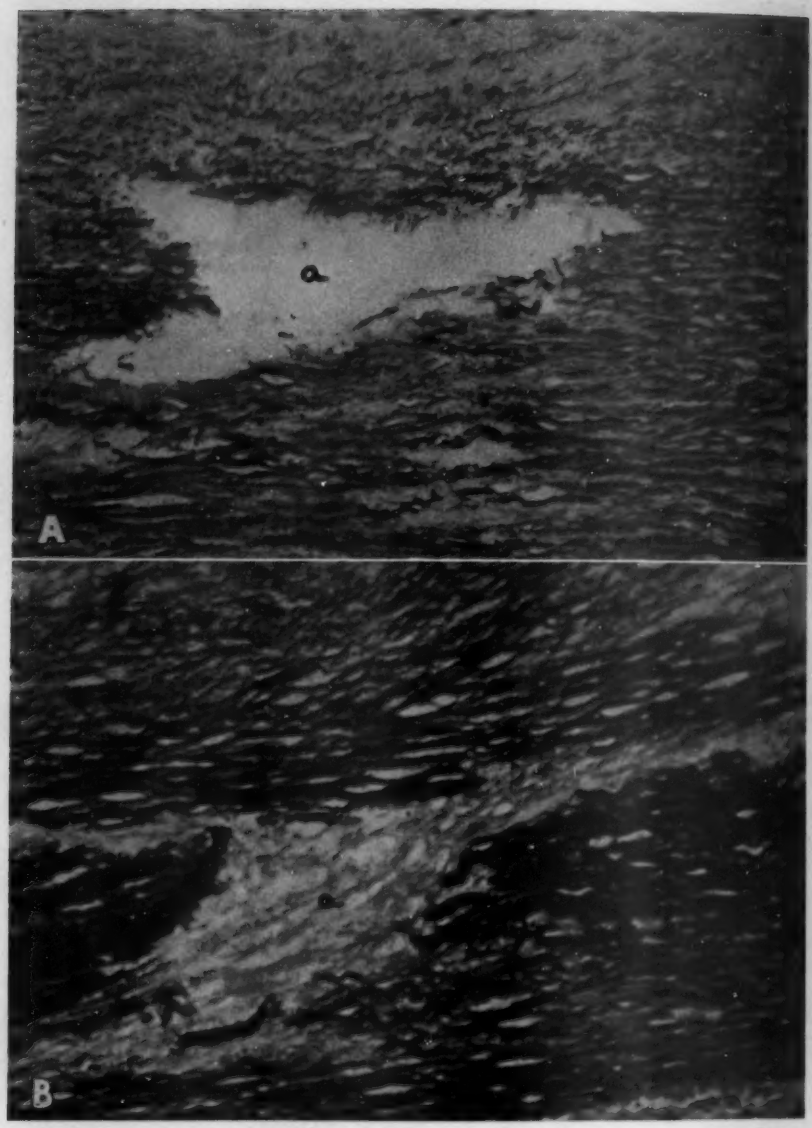

Fig. 2.—*A*, Weigert's elastic tissue stain showing, under low magnification, a cyst (*a*) in the media which with the hematoxylin-eosin stain is seen to contain a basophilic substance. *B*, Weigert's elastic tissue stain showing, under low power magnification, a defect (*a*) in the media. The substance occupying it is largely a loose fibrillar tissue, probably collagen. A basophilic material is present in the interstices of the fibrils.

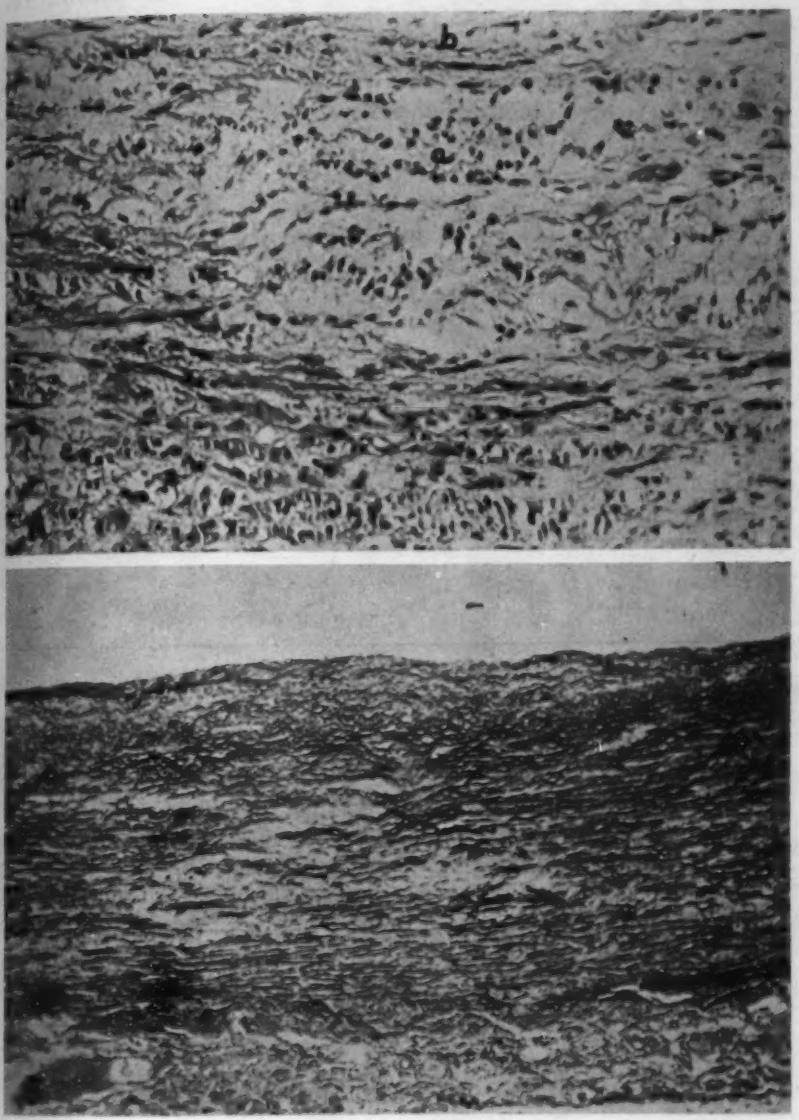

Fig. 3.—*A*, hematoxylin-eosin stain showing, low power magnification, a medial defect occupied by muscle (*a*) the long axis of which lie oblique and perpendicular to that of muscle in normal adjacent media (*b*). *B*, Weigert's elastic tissue stain showing, low power magnification, loss of elastic laminas in medial defects similar to that shown in *A*.

form of organic heart disease were noted to be present sufficiently often to be considered significant. Mindful of this, in the present study I compared the 95 cases in which medial degeneration was present with the remaining 115 in which this lesion was absent.

From the table it will be seen that the lesion is less common in persons below 40 years of age (in 10 of 44, or 20 per cent) than in those beyond 40 (in 85 of 166, or 50 per cent). It was present in 17 of 25 persons beyond 70 years of age. Further, in the younger group significant lesions (2 to 4 plus) occurred in 3 of 10 persons, whereas in the older group such lesions occurred in 40 of 85. Hence it would appear that medial lesions are encountered more often and are of a severer grade in the advanced age group.

Medial Degeneration of Aorta

Age, Yr.	Aortas	Medial Degeneration of Aortas		Aortas in Which Medial Degeneration Was of Given Grade of Severity			
		Absent	Present	+	++	+++	++++
5-10	3	3	0	0	0	0	0
11-20	9	7	2	2	0	0	0
21-30	8	8	0	0	0	0	0
31-40	24	16	8	5	1	0	2
41-50	46	29	17	10	1	2	4
51-60	46	23	23	13	5	1	4
61-70	48	20	28	15	5	2	6
71-80	25	8	17	5	3	1	8
81-85	1	1	0	0	0	0	0
Total	210	115	95	50	15	6	24

To evaluate the age factor, uncomplicated by hypertension and organic heart disease, those cases were selected in which both of these conditions were absent.[7]

There were 67 such cases. Medial degeneration occurred in 23 of them (34 per cent). Of these patients 16 (70 per cent) were above 40 years of age. In 19 of the 23 cases the lesions were only of 1 plus severity. Thus it seems that age continues to be a factor despite the fact that the incidence of lesions and the degree of severity are less.

The effect of rheumatic heart disease and that of hypertension were next studied in 23 and 54 cases, respectively. With regard to rheumatic heart disease, the cases comprising the group studied were those in which gross involvement of one or more valves of varying degrees of severity was shown. Cases in which the process was active and cases in which the disease was inactive were included. In every case gross

7. The cases excluded were those in which there were questionable lesions of the valves, auricular or ventricular endocarditis, pericarditis either recent or old, cardiac enlargement, coronary disease, myocardial fibrosis as determined by gross examination and the study of several microscopic slides, or any evidence of syphilis of the aorta.

observations were substantiated by microscopic examination of one or all valves, mural endocardium, pericardium and myocardium. In 47 of the cases in which hypertension was recorded this was of the essential type, and the usual gross and microscopic changes associated with such a condition were present. The causes of death were chiefly heart failure, cerebral hemorrhage and uremia. In the remaining cases death occurred from miscellaneous causes. Five cases in which blood pressure was unrecorded or was normal or low were included because in these hypertrophy of the heart, nephrosclerosis and arteriolar changes were presented. The patients died either of coronary occlusion with myocardial infarction or from massive cerebral hemorrhage. One additional case was included in which the blood pressure was normal during the patient's stay in the hospital but in which there was a reliable history of repeated periods of hypertension. The final case included in the series was one in which high blood pressure was related to chronic diffuse glomerulonephritis. Cases in which there were unrecorded hypertension and enlargement of the heart but in which arteriolar and renal changes were lacking were not included, so as to escape the criticism of failing to recognize idiopathic hypertrophy. Cases in which hypertension was recorded without concomitant cardiovascular changes were also excluded, since the elevation of blood pressure was not rechecked and in most instances could be ascribed to a terminal cerebral accident.

The effects of other types of heart disease were not studied because the numbers of cases were too few.

There was no indication that the presence of rheumatic heart disease increases the incidence of medial degeneration beyond that of the non-cardiac group. The incidence of medial degeneration in the rheumatic group was 31 per cent (7 of 23 cases). Five of the patients were above 40 years of age. The lesion was of from 2 to 4 plus severity in 3 cases. In the hypertensive group, however, the incidence rises to 51 per cent. Further, there is an increase in the number of cases in which severer grades of medial degeneration were present (17 of 28), with 2 to 4 plus lesions. All patients in this group were above 40 years of age. Unfortunately, there were not, in the hypertensive group, enough patients below 40 years of age to permit study of the effect of elevated blood pressure on such a group.

COMMENT

The lesion of simple muscle loss noted in this study was similar to the medionecrosis observed by Gsell [4a] in spontaneous rupture of the aorta with the exception that necrotic muscle cells were not demonstrable. Cellina,[2] Weise [1] and Moritz [5] describe simple muscle loss in the intact aorta—the first author, in 9 of 10 selected aortas from persons between

71 and 95 years of age; the second, in 9 of 120 specimens unselected in regard to age, and the last author, in 6 of 70. The increased incidence in the present series is due probably to the use of large sections. Whereas Weise [1] and Cellina [2] in their cases both failed to observe more advanced lesions, in my study muscle loss, elastic tissue disintegration and cyst formation were seen in 8 aortas; and a lesion apparently similar to muscle regeneration in the sense of Erdheim was seen in 1 aorta.[8] Scars of the type described by Gsell were noted once.

Inasmuch as medial degeneration produced no macroscopically detectable changes or clinically demonstrable symptoms, its importance in the cases studied must be considered as nil. Despite this, its occasional significance in dissecting aneurysms must not be lost sight of, the latter catastrophe occurring most likely from extensive medial degeneration affecting strategic areas. Its further significance is attested by the fact that in occasional instances changes visible to the naked eye are observed, such as thinning of the wall, replacement of the yellow media by gray scars and finally wrinkling of the intima, suggesting syphilis (Erdheim, 1929).[4b] In 1 case the change led to aneurysmal dilatation.[3]

Much has been written on the causes of medionecrosis. The importance of age is noted by Cellina,[2] for he found medial degeneration in 9 of 10 cases in which all the patients were older than 72 years. The high incidence in the aged is corroborated in the present study. Medial degeneration has been described in aortas from patients with rheumatic fever.[9] It was likewise observed in aortas from persons with rheumatic fever in the present series. However, there was no increase in incidence over that of a control group. It is fully realized, however, that the number of specimens from persons with rheumatic fever in this series is too small to enable one to draw hard and fast conclusions.

The greater frequency of medial degeneration in a group with hypertension gives the latter state etiologic significance. The mechanism can only be conjectured. It may be exhaustion of the contracting muscle in its effort to prevent overdistention of the aorta. It may be the sudden tug on the wall of the vessel caused by abrupt increases in pressure in a vessel already the seat of hypertension. Finally, Erdheim (1930)[4b] offered an explanation on the basis of hyperepinephrinism, a substance which he suggests may be present in excessive amounts in persons with hypertension. This, by causing spasm of the vasa vasorum, may lead to focal ischemia and necrosis.

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8. That the picture seen in this lesion represents muscle regeneration was questioned by Cellina (Cellina, M.: Arch. ital. di anat. e istol. pat. **2**:1105, 1931). He felt instead that it is due to a clumping of the original muscle following loss of support through disintegration of elastic tissue.

9. Klotz, O., and Simpson, W.: Am. J. M. Sc. **184**:455, 1932. Pappenheimer, A. M., and Von Glahn, W. C.: ibid. **44**:489, 1924

SUMMARY

Medial degeneration was noted in 95 of 210 aortas from routine autopsy material. In 92 of these the lesion was a simple loss of muscle. In 8 aortas only were advanced changes seen, such as loss of elastic tissue and cyst formation.

In 67 vessels from persons without organic heart disease or hypertension the incidence of medial degeneration was 34 per cent. The lesions were of mild degree. Seventy per cent of the patients were above 40 years of age.

In 23 aortas from patients with rheumatic heart disease the incidence of medial degeneration was 31 per cent. In 54 aortas from patients with hypertension the incidence rose to 51 per cent. Also, the number with severe grades was greater.

Case Reports

MULTINUCLEATE EPITHELIAL GIANT CELLS WITH INCLUSION BODIES IN PRODROMAL MEASLES

Report of an Autopsy

K. H. Semsroth, M.D., Amsterdam, N. Y.

Amitotic giant cells form a characteristic response of the host cells to some of the virus diseases, as pointed out by Rivers.[1] In the case of measles, a disease generally believed to be due to a filtrable virus, multinucleate giant cells have been found in the lymphatic organs. Since Warthin,[2] in 1931, pointed out the pathognomonic nature of these cells, they have been observed [3] as early as five days prior to and [3c] as late as two days after the appearance of the exanthem. Lesions of the respiratory tract, equally characteristic for measles, had not been described until Masugi,[4] in 1938, reported on the occurrence of epithelial multinucleate giant cells with cytoplasmic inclusion bodies in the pharynx, trachea and bronchi four days after the appearance of the exanthem. In prodromal measles, pathognomonic changes other than those of the lymphatic organs have as yet not been described. Such lesions, observed in the respiratory tract as well as in the lymphatic apparatus prior to the outbreak of the exanthem, are the subject of the present report. Permission to use the clinical records of this case was given by Dr. S. L. Homrighouse, of Amsterdam, N. Y.

REPORT OF A CASE

A boy 20 months old was twice exposed to measles in the course of two weeks. About fourteen days after the first exposure he began to suffer from acute, severe dyspnea and died of respiratory difficulty two hours after an unsuccessful attempt at bronchoscopic examination. At autopsy three small erosions of the laryngeal mucosa were seen besides edema and congestion of the trachea and large bronchi. With these extremely scanty gross changes, the case remained unexplained until sections of the respiratory tract and lymphatic organs revealed lesions indicative of measles.

From the Montgomery County Laboratories, Amsterdam, N. Y., and the Department of Pathology, Albany Medical College, Albany, N. Y.

1. Rivers, T. M.: Am. J. Path. **4**:91, 1938.

2. Warthin, A. S.: Arch. Path. **11**:864, 1931.

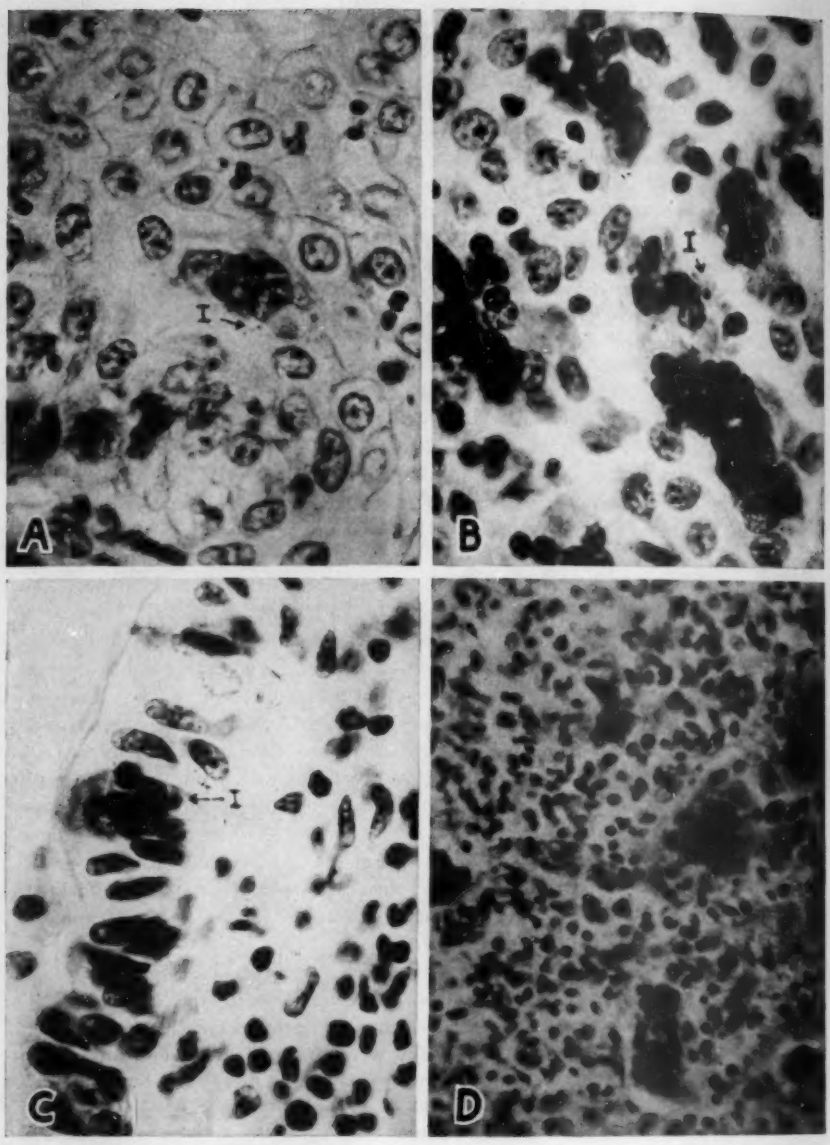
3. (*a*) Finkeldey, W.: Virchows Arch. f. path. Anat. **281**:323, 1931; (*b*) **284**:518, 1932. (*c*) Davidsohn, J., and Mora, J. M.: Arch. Path. **14**:757, 1932. (*d*) Herzberg, M.: J. A. M. A. **98**:139, 1932. (*e*) Fischer, W.: Beitr. z. path. Anat. u. z. allg. Path. **91**:474, 1933. (*f*) Schultze, W. H.: München. med. Wchnschr. **80**:576, 1933. (*g*) Hathaway, B. M.: Arch. Path. **19**:819, 1935. (*h*) Graeff, S.: Deutsche med. Wchnschr. **63**:1357, 1937.

4. Masugi, M., and Minami, G.: Beitr. z. path. Anat. u. z. allg. Path. **101**: 483, 1938.

Retrogressive epithelial changes were seen at the base of the tongue and throughout the respiratory passages. In all of these locations vacuolation, occasional pyknosis and desquamation of epithelial cells were present. Lymphocytes and, in lesser number, polymorphonuclear leukocytes were migrating through the epithelium. In the larynx and trachea the degenerative epithelial changes had progressed to the formation of erosions. In the epithelium of the tongue one found an occasional multinucleate giant cell, with a cluster of three or four centrally situated nuclei (*A* in figure). In some instances there was contained in the cytoplasm a group of faintly basophilic globoid bodies. No mitoses were seen. Many such amitotic giant cells were seen in the epithelium of the tonsillar crypts (*B* in figure). Small globoid inclusion bodies occurred in their cytoplasm. In this location they were usually eosinophilic. The oral mucosa and the mucosa of the entire upper respiratory tract were diffusely infiltrated with many mononuclear round cells and few polymorphonuclear leukocytes. Plasma cells predominated around the mucous glands of the trachea and in the larynx. Some of them contained two or three nuclei; others, a cluster of five or more, surrounded by somewhat ill defined cytoplasm—a picture suggesting the formation of multinucleate giant cells through amitotic division of the nuclei of plasma cells.

The epithelium of the bronchi showed vacuolation and desquamation, with complete loss of the surface epithelium in places, particularly over lymphoid nodules. An outstanding feature was the presence of multinucleate giant cells in the epithelium (*C* in figure). A cluster of six or more hyperchromatic nuclei could be seen in an epithelial cell, which might or might not retain its cilia. Some of the giant cells contained eosinophilic inclusion bodies, usually globoid or oval. As a rule, they were situated at the basal side of the cluster of nuclei. They were not seen in other epithelial cells. In some giant cells fusion of nuclei had led to the formation of large polylobate masses of chromatin, found within the epithelium or on its surface. The epithelium of the bronchial glands also showed an occasional multinucleate giant cell. No mitoses were seen. Mononuclear round cells, few polymorphonuclear leukocytes, plasma cells and multinucleate plasma cells infiltrated the bronchial mucosa. The lungs proper showed peribronchiolitis, with predominance of mononuclear round cells. Adjoining interalveolar septums were frequently thickened through an increase in the number of their nuclei. Occasionally, large polylobate masses of chromatin were seen in alveolar lumens. Some of them showed cytoplasm provided with cilia, obviously aspirated bronchial epithelium. Here and there a cell of the alveolar lining showed a cluster of hyperchromatic nuclei. Interlobular edema was marked.

Of the lymphatic organs, the palatine tonsils, peribronchial and hilar lymph nodes, spleen and thymus were examined. The tonsils were the seat of inflammatory hyperplasia. The germinal centers showed retrogressive changes. Within them numbers of multinucleate giant cells were present (*D* in figure). Their densely crowded hyperchromatic nuclei—up to twelve or more—left but a narrow margin of cytoplasm. The same type of giant cell was present in peribronchial and hilar lymph nodes, with lymphoid hyperplasia and retrogressive changes of germinal centers. It was also seen in many lymph follicles of the spleen and was apt to be located near the periphery of the germinal center. Lymphocytes and few eosinophilic round cells infiltrated the stroma of the thymus. Its parenchyma contained large numbers of multinucleate giant cells similar to those found in the tonsils and lymph nodes. The number of their small hyperchromatic nuclei varied from three or four to fifty or more. Within the largest ones fusion of



A, epithelium at the base of the tongue. At *I* is seen a multinucleate epithelial cell with cytoplasmic inclusion bodies. *B*, multinucleate giant cell with inclusion bodies (*I*) in epithelium of the palatine tonsils. *C*, multinucleate giant cell in epithelium of a large bronchus. At *I* an eosinophilic inclusion body is seen, surrounded by a clear zone. *D*, palatine tonsil showing multinucleate giant cells in germinal centers of the lymphatic tissue.

nuclei into bizarre-shaped masses of chromatin had frequently occurred. No mitoses were found in thymus, tonsils or lymph nodes. Extensive phagocytosis of nuclear fragments by reticulum cells was observed.

SUMMARY

In a fatal case of prodromal measles with multinucleate giant cells in the lymphatic organs and thymus, certain lesions of the respiratory tract were observed which were considered characteristic. In the epithelium of the respiratory passages, degenerative changes were associated with proliferative ones. The latter consisted in the appearance of amitotic multinucleate epithelial cells comprising cytoplasmic inclusion bodies. In the underlying mucosa, amitotic division of plasma cells accounted for the formation of some multinucleate giant cells.

PAPILLARY TUMOR OF THE CHOROID PLEXUS IN A NEWBORN INFANT

GERHART A. DRUCKER, M.D., BROOKLYN

In recent years the number of reported cases of papilloma of the choroid plexus has considerably increased. However, this type of tumor is still regarded by most authors as one of the rarest among all types of cerebral neoplasms. Cushing's [1] series of more than 2,000 verified cases of intracranial tumor contains 12 of papilloma of the choroid plexus, an incidence of 0.6 per cent. Van Wagenen,[2] who summarized the literature on this subject in 1930, collected 45 cases from the literature and added 2 of his own. In 1936 Friedman and Solomon [3] published a comprehensive paper on cases of tumor of the choroid plexus in childhood. Reviewing the previous literature, they were able to find 34 true cases in all age groups, including 1 case of their own. In 14 of these cases the patient had been an infant or a child. Another review of this subject, published by Turner and Simon [4] in 1937, mentions 72 reported cases. Since the publication of the latter review, several new case reports have appeared in the literature.

Papillary tumor of the choroid plexus has been found in all age groups, but the incidence is highest in infants and children. The youngest patient in all previously reported cases was a boy 10 weeks of age.[5] The case presented in this paper is remarkable because a papilloma of the choroid plexus was found at autopsy in a newborn infant.

REPORT OF A CASE

Mrs. A. M., aged 28, was admitted to the maternity service of the Israel Zion Hospital on Feb. 14, 1938, in active labor. This pregnancy was her second. The first child was 6 years of age, normally developed and in good health. The family history was entirely irrelevant. A diagnosis of frank breech presentation was made, and the child was delivered by breech extraction. It was deeply cyanotic and showed no sign of life. The usual measures of resuscitation were applied but were not successful.

Postmortem Examination.—The body was that of a full term newborn boy measuring 54 cm. in length and weighing 3,530 Gm. The skull was larger than is usual; the circumference of the suboccipitobregmatic plane measured 36.5 cm.; that of the mentooccipital plane, 44 cm. Both fontanels were bulging slightly. The greater fontanel admitted the tips of two fingers; the lesser, the tip of one

From the Department of Pathology of the Israel Zion Hospital.

1. Cushing, H.: Intracranial Tumors, Springfield, Ill., Charles C. Thomas, Publisher, 1932.

2. Van Wagenen, W. P.: Arch. Surg. **20**:199, 1930.

3. Friedman, J. J., and Solomon, C. I.: Am. J. Dis. Child. **52**:114, 1936.

4. Turner, O. A., and Simon, M. A.: Am. J. Cancer **30**:289, 1937.

5. Weinstein, E. A.: J. Mt. Sinai Hosp. **5**:573, 1938.

finger. The cranial bones touched each other at the sutures. The face was deeply cyanotic, and the eyelids were edematous. All signs of maturity were present.

The scalp was markedly edematous. On opening the cranial cavity, there escaped approximately 100 cc. of nonclotted blood. One noted a complete tear of the right half of the tentorium cerebelli and a partial tear of the vena cerebri magna. A large amount of loosely clotted blood was present in the subdural space and below the tentorium.

The brain measured 15 by 13 by 9.5 cm. and weighed 310 Gm. The leptomeninges in large areas were hemorrhagic. There was evident at once marked atrophy of the cortex of the left parietal and occipital lobes, which showed no convolutions and no sulci. Palpation of this area revealed great flabbiness of the brain tissue, beneath which, however, a mass of firmer consistency was felt. Removal of a thin shell of brain tissue in this area exposed a well outlined ovoid tumor, which measured 4 by 3.5 by 3 cm. (fig. 1). It was located within the greatly dilated posterior horn of the left lateral ventricle and was firmly attached

Fig. 1.—Lateral view of the left cerebral hemisphere. The thinned out cortex of the parietal and occipital lobes has been removed and the tumor within the greatly dilated posterior horn of the left lateral ventricle is exposed.

to its floor, medial wall and roof. The choroid plexus, coming from the inferior horn, entered the tumor at the junction of the inferior and posterior horns. Two large blood vessels, coming from the plexus, entered the tumor at this place. Further dissection showed that the wider of these two vessels was a direct continuation of the greatly dilated anterior choroid artery. The tumor was lined by a very thin, delicate, wrinkled fibrous capsule, rich in blood vessels near the junction of the choroid plexus and the growth. The surface of the growth was pale yellowish gray and showed deep furrows, along which the capsule was firmly attached to the underlying tumor tissue. In other places, particularly where the growth was fixed to the walls of the ventricle, a free space remained between the tumor tissue and its capsule. In these areas the capsule, on superficial examination, seemed to merge with the ependyma of the ventricle.

The cut surface of the neoplasm appeared papillary, soft and grayish pink. Many small papillae also originated from the inner lining of the fibrous capsule.

The cortex of the left parietal and occipital lobes and the underlying white matter were compressed to a shell 0.5 cm. in thickness. Similar compression and atrophy, with loss of the normal convolutions, were seen on the medial aspect of the left occipital lobe. The anterior and inferior horns and the central part of the left lateral ventricle were normal in width. The optic thalamus was in front of the tumor and not compressed by it. The lentiform nucleus had no relation to the tumor. The remaining portions of the ventricular system were normal in width and lined by smooth, pale ependyma. The foramina of Monro, Magendie and Luschka were patent.

The organs of the neck showed nothing unusual. The lungs were strikingly emphysematous, undoubtedly as a consequence of the mouth to mouth breathing which had been done as a last effort of resuscitation. Along the edges of both

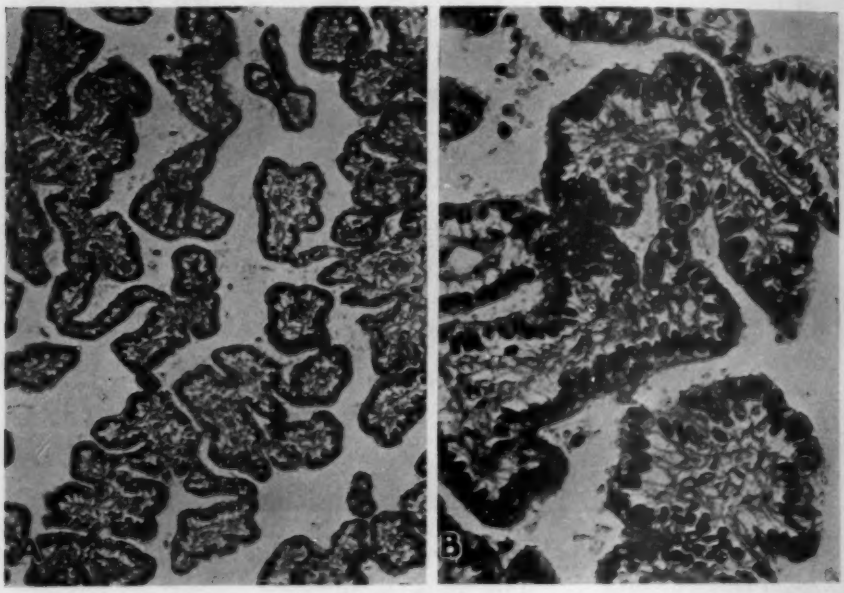


Fig. 2.—Section of papilloma of the choroid plexus; hematoxylin and eosin stain. *A*, low magnification; *B*, high magnification.

lungs were many thin-walled emphysematous blebs, some of them 0.5 cm. in diameter. The pleura was smooth and shiny throughout. There were many subpleural petechial hemorrhages. The free and the cut surfaces of both lungs were bright pink, with only a few small dark areas of atelectasis interspersed. The heart was normal save for numerous subepicardial petechial hemorrhages, 1 to 2 mm. in diameter. The remaining organs showed nothing unusual.

Microscopic Examination.—Sections of the tumor showed an architecture of innumerable delicate dendritically branching papillae, each consisting of a core of vascularized connective tissue and lined by a single layer of tall columnar epithelium (fig. 2*A*). The papillae originated from a scaffolding of dense connective tissue, which in some places formed cystlike spaces enclosing a great number of tumorous papillae. The lining epithelium showed a regular palisade-like arrangement. Each epithelial cell contained a round or spindle-shaped, moderately

hyperchromatic nucleus with numerous fine chromatin granules. In most areas each nucleus was in the peripheral half of the epithelial cell, leaving only a narrow strip of cytoplasm between its peripheral pole and the free surface of the cell. In other places the nucleus occupied the center or the basal portion of the epithelial cell. The cytoplasm of the epithelial cells was eosinophilic and granular in its peripheral half, whereas its basal portion was clear, showing the cell border distinctly. On their free surface many of the epithelial cells carried short delicate cilia, among which sometimes one solitary long cilium was seen. A real brush border was present in very few places. Blepharoplasts were absent in all sections.

The stroma of the papillae consisted of rather loose, wavy connective tissue, which stained blue with Mallory's aniline blue–orange gold stain.[5a] The larger stromal septums contained many thin-walled arterioles and capillaries. Loosely scattered in between were fibroblasts, occasional lymphocytes and, in moderate numbers, large clear mesenchymal cells. The smallest papillae consisted only of two layers of epithelium, sometimes arranged around a wide capillary. A colloid-like degeneration of the stroma was seen in many places; these areas stained bright red with Mallory's aniline blue–orange gold stain. Some of these degenerated papillae were markedly swollen and lined by cuboidal epithelium.

The capsule of the tumor consisted of a very thin layer of collagenous connective tissue, lined on the inside by papilliferous epithelium of the same type as that composing the tumor tissue itself. In many places bizarre papillomatous formations arising from the inner outline of the capsule protruded into the tumor tissue. Sections of those areas where the capsule was adherent to the ventricular wall showed an irregular furrow-like protrusion of the capsule into the surrounding brain tissue. In some places the capsular epithelium covered the underlying brain tissue so closely as to resemble an ependymal lining. By proper staining methods, however, the collagenous connective tissue of the capsule could be clearly demonstrated everywhere as a thin layer between the epithelium and the underlying brain tissue, proving beyond doubt that the former originated from the choroid plexus and not from the ependyma. Mitotic figures and atypical cell forms were absent in all sections.

Sections of the lungs revealed full aeration and marked emphysematous distention of all alveoli. The other organs showed no important histologic changes.

Diagnosis.—The diagnosis was: papilloma of the choroid plexus, located in the posterior horn of the left lateral ventricle of the brain; atrophy of the left parietal and occipital lobe; tear of the tentorium cerebelli and vena cerebri magna with massive subtentorial hemorrhage; acute diffuse pulmonary emphysema (the latter probably due to the attempts at resuscitation, including mouth to mouth breathing); multiple subpleural and subepicardial petechial hemorrhages.

COMMENT

This is the first reported case of papilloma of the choroid plexus in a newborn infant. However, several cases have been reported in which such a tumor was found in very young infants, and one is justified in assuming that in these cases the growth had been present at birth. This is particularly true of those cases in which an enlargement of the head was noted at birth or a few weeks afterward. In the case presented,

5a. Mallory, F. B.: Pathological Technique: A Practical Manual for Workers in Pathological Histology, Philadelphia, W. B. Saunders Company, 1938, p. 153.

death was not caused directly by the tumor itself, but by a birth injury inflicted during the delivery of the aftercoming head. On the other hand, the tumor must be regarded as the indirect cause of death, since the enlargement of the head caused by the growth certainly predisposed to a tear of the tentorium, especially in breech presentation. Had the child survived its birth, neurologic symptoms would probably have been present from the very onset, in view of the size of the tumor and of the amount of compression of the adjacent structures that was already present.

Microscopically, the neoplasm showed all the characteristics of a papilloma of the choroid plexus. The absence of atypical changes and mitotic figures in the lining epithelium let it appear as a benign growth. On the other hand, an irregular protrusion of the capsular epithelium into the surrounding brain tissue was seen in several places, which seems to speak for an invasive character of the growth.

Since the tumor was adherent to the wall of the ventricle and since in some of these areas the papilliferous epithelium formed a lining of the underlying brain tissue resembling ependyma, the question arose whether this neoplasm originated from the ependyma or from the epithelium of the choroid plexus. Conclusive evidence of its origin from the latter is furnished by the transition of the choroid plexus into the neoplasm and by the collagenous connective tissue stroma of the growth. Moreover, the presence of a thin layer of collagenous connective tissue between the capsular epithelium and the brain tissue in all those places where the capsule was adherent to the ventricular wall proved conclusively that this epithelial lining was not identical with ependyma.

Mallory pointed out a valuable histologic point of differentiation between ependymoma and other types of glioma, namely, the presence of blepharoplasts in the former. Davis and Cushing,[6] Van Wagenen[2] and others used the same method to differentiate papillary ependymoma from papilloma of the choroid plexus. Blepharoplasts were absent in the case described here, although many epithelial cells carried cilia on their free surface. The latter fact does not speak against the origin of the tumor from the choroid plexus, since the epithelium of the choroid plexus is normally ciliated during fetal life, the time of origin of this neoplasm.

A comparison of all reported cases of papilloma of the choroid plexus in young infants shows a striking similarity of the essential features in almost all cases. Thirteen cases of this tumor, including the one reported here, have been described in which the patients were infants up to 2 years of age.[7] In 9 of these cases, the left lateral ventricle

6. Davis, L. E., and Cushing, H.: Arch. Neurol. & Psychiat. **13**:681, 1925.

7. (*a*) Graves, G. W., and Fliess, M. M.: Am. J. Dis. Child. **47**:97, 1934. (*b*) Toeppich, G.: Frankfurt. Ztschr. f. Path. **33**:238, 1925. (*c*) Bleyer, A., and Siebert, W. J.: J. Pediat. **8**:193, 1936. (*d*) Stroeber, H.: Berl. klin. Wchnschr. **30**:123, 1893. (*e*) Goodhart, G. W.: Guy's Hosp. Rep. **69**:217, 1918. (*f*) Weygandt: Deutsche med. Wchnschr. **43**:797, 1917. (*g*) Noodt, K.: Virchows Arch. f. path. Anat. **258**:331, 1925. (*h*) Ebbs, J. H.: Arch. Dis. Childhood **12**: 403, 1937. (*i*) Van Wagenen.[2] (*j*) Friedman and Solomon.[3] (*k*) Turner and Simon.[4] (*l*) Weinstein.[5]

was the seat of the growth; in 3, the right lateral ventricle, and in 1, the fourth ventricle. Marked hydrocephalus was present in 11 cases. The reports of 5 cases mention marked atrophy of the cerebral cortex, with flattening of the gyri over the seat of the tumor. The average size of the growths was 3 to 5 cm. in diameter, but some had a diameter of 7 to 8 cm. The tumors were usually reddish brown, soft, friable and well outlined from the surrounding structures. In 5 cases, including the present case, the tumor was fixed to the wall of the ventricle. Four reports mention encapsulation of the neoplasm, while two others emphasize the absence of a capsule. Grossly visible hemorrhage from the tumor tissue was present in 3 cases, in 1 of which it was the immediate cause of death.

Microscopically, in all cases the neoplasm imitated more or less closely the structure of the choroid plexus. In all cases the predominant histologic picture was that of delicate branching papillae, each consisting of a core of vascularized connective tissue, lined by a single layer of columnar epithelial cells. The histologic picture in most cases was that of a benign, nonaggressive growth. In 2 cases, however, the neoplasm showed definite evidence of malignancy: the case published by Graves and Fliess [7a] was that of an anaplastic growth fixed to the brain tissue; Toeppich's case [7b] was that of a tumor completely filling out the fourth ventricle of a 2 year old child with definite atypism of the epithelial cells and numerous metastases throughout the brain and the spinal cord. In 2 cases the classification of the growth as benign or malignant is doubtful, since the neoplastic epithelium, while free from atypical changes, had invaded the brain tissue to a slight extent; these are the cases reported by Bleyer and Siebert [7c] and that reported in this paper. The former also showed a few "seed implants" in the vicinity of the tumor mass. Such implants, according to most authors, are not a sign of malignancy. Finally, the tumor described by Friedman and Solomon [8] is worth mentioning because of the presence of many multinucleated giant cells and the lack of uniformity of its epithelial cells. This neoplasm was not encapsulated and was attached to the floor of the descending horn by a broad base. Its surface was eroded and a massive hemorrhage had occurred from it.

Degeneration and swelling of some papillae were present in almost all cases. In Stroeber's [7d] case these degenerated papillae could be seen grossly as pea-sized gelatinous spheres embedded in tumor tissue.

SUMMARY

A case of papilloma of the choroid plexus in the left lateral cerebral ventricle of a newborn infant has been described; the tumor showed no histologic signs of malignancy but had invaded the brain tissue to a slight degree. The reported cases of papilloma of the choroid plexus in young infants are strikingly similar in their essential features.

Laboratory Methods and Technical Notes

USE OF SOLID CARBON DIOXIDE IN PREPARATION OF GROSS PATHOLOGIC SPECIMENS

JOHN T. BAUER, M.D., PHILADELPHIA

With the commercial introduction of solid carbon dioxide ("dry ice") in 1925, methods were soon devised for using it in the preparation of frozen sections for microscopic examination,[1] but so far as I am aware no mention has been made of its application in the preparation of gross specimens. For those lacking suitable refrigeration who occasionally wish to prepare longitudinal or cross sections of large parts of cadavers, such as an extremity, in such a manner as to preserve the relationships and color of the bones and soft parts, the following methods can be recommended.

A fiber packing box or container large enough to hold the part to be frozen is obtained and lined with an old woolen blanket and a few newspapers. On this the specimen is placed, and then small pieces of solid carbon dioxide (5 to 25 cm. in diameter) are placed about the specimen until it is entirely surrounded. (Heavy gloves should be worn while cracking the solid carbon dioxide and packing it about the specimen and while handling the specimen after it is frozen, to prevent severe frostbite.) The newspapers and blanket are then tightly wrapped about the solid carbon dioxide.

Depending on the temperature of the room and the size of the specimen, a block or two of solid carbon dioxide (50 to 100 pounds [22.7 to 45.4 Kg.]) will suffice to freeze an arm or a leg solid within twenty-four to thirty-six hours. At the end of this time gross sections can be sawed in any plane through skin, subcutaneous fat, muscles, blood vessels and bone, without tearing or distorting of the tissue. (We have used a carpenter's crosscut saw with 8 or 9 teeth per inch.) The sawdust that adheres to the cut surface of the specimen is quite dry at first and can be scraped away with the edge of a knife.

If the cut surface is then gently and quickly washed with cold water, the water will be frozen into a thin smooth transparent layer of ice, which readily permits photography with few or no dazzling highlights or reflections. Color photographs of such specimens are especially pleasing, as the natural colors are preserved.

Blocks can then be taken from the frozen specimen with a saw and chisel and placed in the usual histologic fixatives for the preparation of material for microscopic study. No detrimental effect other than slight shrinkage has been noted in

From the Ayer Clinical Laboratory of the Pennsylvania Hospital.

1. Dunn, F. L.: J. Lab. & Clin. Med. **16**:627, 1931. Lindsay, J. W.; Rice, E. C., and Selinger, M. A.: J. A. M. A. **96**:773, 1931.

the microscopic sections of tissue frozen by this means. Although chemical analyses of tissue frozen by solid carbon dioxide have not been made in this laboratory, they should be possible, as rapid freezing retards most chemical changes.

Modifications of the method outlined have been suggested but not tried. These include injection of the vascular bed of the specimen with fixatives before freezing and use of a band saw or other kinds of power saws instead of a hand saw.

The cost is little more than that of the solid carbon dioxide, which in our experience was between $3 and $3.50 per specimen.

The method described has been used in this laboratory on three occasions. On two, the specimen was a lower extremity removed at the hip joint because of an osteogenic sarcoma of the femur, and on the third occasion the specimen was an upper extremity removed at the

An upper extremity removed at the shoulder from a woman suffering with osteitis deformans (Paget's disease) associated with an osteogenic sarcoma of the lower end of the humerus.

shoulder from a woman suffering with osteitis deformans (Paget's disease) associated with an osteogenic sarcoma of the lower end of the humerus. A photograph of this specimen (figure) demonstrates the usefulness of the method by illustrating (1) the preservation of the relationship of the tumor in the humerus to the surrounding soft parts and (2) the almost complete absence of disturbing reflections usually seen in fresh tissue.

SUMMARY

A satisfactory method is outlined whereby gross sections of tissue can be prepared by sawing through the soft parts and bone after the entire specimen has been frozen with solid carbon dioxide ("dry ice").

A SIMPLE METHOD OF PREPARING LANTERN SLIDES OF HISTOLOGIC SPECIMENS IN TWO COLORS

TRACY J. PUTNAM, M.D., BOSTON

The preparation of lantern slides in colors resistant to heat has become a simple matter since the introduction of kodachrome film in cut film sizes. A film 2¼ by 3¼ inches (5.7 by 8.2 cm.) is large enough to fit into the mat of an ordinary slide, or two 35 mm. films may be mounted on the same slide. There are still three objections to kodachrome, however: One is the matter of expense, which precludes its routine use in many laboratories. The second is that exposure must be accurate within narrow limits. Third, the success of the photograph can be judged only after the film has been returned from the processing plant.

An easy and inexpensive method of approximating the colors of microscopic slides stained with hematoxylin-eosin, methylene blue-eosin, polychromatic methylene blue or its substitutes, Mallory's connective tissue stain, Mann's stain and others in which the only tints represented are blue and light shades of red is to tone, mordant and stain an ordinary lantern slide plate. The method is borrowed from a method once used in the moving picture industry.[1] Blue or brown toning of slides is, of course, an old trick, and Jones [2] recently called attention to the usefulness of mordanted colors for lantern slides of histologic material.

The method is as follows:

A soft, well exposed negative is taken on fine grain panchromatic film with a Wratten E (orange) filter, at any desired enlargement. From the negative a soft positive lantern slide is prepared. It should be of such quality that practically no blacks are present. The blues should be represented by dark grays; reds and pinks, by extremely light tones. There should be some areas in the slide entirely free from deposits of silver, corresponding to empty spaces in the section. A weak ferricyanide bleach may be used to obtain these white spaces if necessary. The slide should be thoroughly fixed in fresh sodium thiosulfate (hyposulfite) and

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From the Neurological Unit, Boston City Hospital, and the Department of Neurology, Harvard Medical School.

This method was developed in the course of investigations financed by the Multiple Sclerosis Fund of Harvard University, including a grant from the John and Mary Markle Foundation.

1. Tinting and Toning of Eastman Positive Motion Picture Film, ed. 4, Rochester, N. Y., Eastman Kodak Co., 1927.

2. Jones, W. R.: J. Lab. & Clin. Med. **23**:1297, 1938.

hardener—best in two successive baths. It is then washed for one or two hours in running water. Incomplete fixation or the slightest trace of sodium thiosulfate is ruinous.

The slide is then toned in the following solution (Eastman formula T-18):

Ammonium persulfate	1 Gm.
Ferric alum (ferric ammonium sulfate)	2.5 Gm.
Oxalic acid	6 Gm.
Potassium ferricyanide	2 Gm.
Hydrochloric acid (10 per cent)	2 cc.
Water to make	2,000 cc.

The slide should be toned until the dark grays are blue, leaving light tones mordanted but unchanged in color. It is often well to leave a few points still black. It is washed ten to fifteen minutes (not longer) and then immersed in the following solution (Eastman formula T-17a):

Safranin[3]	0.2 Gm.
Acetic acid (glacial)	0.5 cc.
Water to make	1,000 cc.

It is left in this solution until the light tones acquire the desired color (usually fifteen minutes). The blue may be intensified by washing and returning it to the iron bath, and the red may be made deeper by returning it to the safranin bath. Overstaining with safranin may be remedied by dipping in 0.2 per cent solution of concentrated ammonia, then washing, but there is no way of decreasing the intensity of the blue.

When the colors are satisfactory, the slide is washed for ten minutes and dried. A little yellow may be "dubbed in" if desired. The finished slide is given a coat of varnish and mounted in the usual fashion. The colors are permanent, and the slide is resistant to heat. The reds produced are somewhat brownish, but the lighter shades are satisfactory, and the effect is convincing.

For other combinations of colors which might be useful—for example, blue and yellow for Weigert-Pal slides; brown and yellow for silver stains—consult the manual on toning motion picture films.[1]

SUMMARY

A simple and inexpensive method of combined toning, mordanting and staining of ordinary lantern slide positives is described, which is applicable to histologic subjects stained by methods in which blues and reds alone are represented.

3. Quantities under 1 pound (453 Gm.) may be obtained from the Eastman Kodak Company, Rochester, N. Y. Larger amounts from the National Aniline Company, 40 Rector Street, New York.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Normand L. Hoerr, assistant professor of anatomy in the University of Chicago, has been appointed professor of anatomy in Western Reserve University, succeeding the late Thomas Wingate Todd.

Thomas B. Turner has been appointed professor of bacteriology in the school of hygiene and public health of Johns Hopkins University.

Raymond B. Allen, dean of Wayne University College of Medicine, Detroit, has been appointed executive dean of the Chicago colleges (medical, dental and pharmacal) of the University of Illinois.

Frederick P. Gay, professor of bacteriology in the College of Physicians and Surgeons of Columbia University, died on July 14, at the age of 65.

Stanley P. Reimann, Philadelphia, has been appointed chairman and pathologist of the newly established division of cancer control in the state department of health of Pennsylvania.

Alfred N. Richards, professor of pharmacology, has been appointed to succeed the late Alfred Stengel as vice president of the University of Pennsylvania in charge of medical affairs.

Elson B. Helwig and William O. Russell have been appointed instructors in pathology in Washington University School of Medicine.

Awards.—The Theobald Smith medal and prize of $1,000 has been awarded by the American Association for the Advancement of Science to Albert B. Sabin, associate of the Rockefeller Institute for Medical Research, in recognition of his work on pneumococcic infections.

The annual award of the American Pharmaceutical Manufacturers' Association was presented to Nathan B. Eddy and Lyndon F. Small, of the United States Public Health Service, at the recent annual meeting, in recognition of the "fundamental and outstanding studies of the chemistry, pharmacology and therapeutics of morphine derivatives for the alleviation of human suffering."

The French League against Cancer has awarded the Amerongen prize of 160,000 francs to A. H. Roffo, director of the Institute of Experimental Medicine for the Study and Treatment of Cancer at the University of Buenos Aires.

The Alvarenga Prize of the College of Physicians of Philadelphia has been awarded to Harry Goldblatt for his work on the genesis of hypertension.

Beit Memorial Fellowships for Medical Research, London.—The first Beit fellow, now Sir Thomas Lewis, was elected in 1910. With the elections of 1938, the total of fellows reached 200; of these 30 were women. Of the 170 men, 6 have been elected to the Royal Society and 34 have been appointed university professors. The value of the fellowships varies from $2,000 to $2,500, approximately. The most frequent places of work by Beit fellows have been Cambridge University, London University and the Lister Institute of Preventive Medicine.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Trypsin Injection into Renal Arteries. M. Friedman and L. N. Katz, J. Exper. Med. **68**:485, 1938.

Trypsin injected into both renal arteries of the dog was found to cause acute necrosis of large sections of the kidney, an immediate excretory insufficiency and transient hypertension. In dogs surviving the acute phase of the reaction to the injected trypsin a chronic renal excretory insufficiency developed, with no hypertension, despite the severity and duration of the renal excretory insufficiency. The application of a Goldblatt clamp to the renal artery of one of the two kidneys into which trypsin had been injected led to a rise in blood pressure, which returned at once to normal when the ischemic kidney was removed, even though the preexisting renal excretory insufficiency was augmented. This experience demonstrated unequivocally that chronic renal excretory insufficiency and hypertension are not directly related. The application of a Goldblatt clamp to the renal artery of one kidney and the simultaneous injection of trypsin into the other led to hypertension. The later removal of the ischemic kidney led to severe renal excretory insufficiency; at the same time the preexisting hypertension disappeared. This indicated again that renal excretory insufficiency and renal ischemia produce different phenomena and that the former had no direct relation to hypertension.

From Authors' Summary.

A New Method of Producing Renal Insufficiency and Hypertension in the Rabbit. D. R. Drury, J. Exper. Med. **68**:693, 1938.

A simple method is described for producing renal insufficiency in rabbits by limiting the blood flow through one kidney and later removing the other. Renal insufficiency of any desired degree of severity can be obtained. The limitation of blood flow should be produced by allowing the artery to grow up to the size of a loop of thread laid around it when the rabbit is small. Attempts to reduce the blood flow by constricting the renal artery in the adult rabbit usually result in necrosis of the kidney. The induction of renal insufficiency is usually followed by loss of weight and disturbance of the apparatus for maintaining equilibrium. Hemorrhages into the intestinal wall and into the lumen of the intestine are frequent. A moderate degree of anemia, as judged from hematocrit readings, comes on very rapidly after reduction of the total kidney mass. The total red blood cell volume seems to assume a lower level, which is maintained for a long time. Hypertension is present before the ablation of the right kidney and becomes more severe after this operation. A certain degree of hypertension can be produced in the adult rabbit by moderate restriction of the renal blood flow of one kidney. The severity of this is increased by removal of the other kidney.

From Author's Summary.

Nutritional Cytopenia in the Monkey. W. C. Langston, W. J. Darby, C. F. Shukers and P. L. Day, J. Exper. Med. **68**:923, 1938.

Young rhesus monkeys (Macaca mulatta) were given a diet containing casein, polished rice, whole wheat, salt mixture, sodium chloride, cod liver oil and ascorbic acid. They showed a syndrome of anemia, leukopenia and loss of weight. Ulcer-

ation of the gums and diarrhea were common, and death occurred between the twenty-sixth and the one hundredth day. Four monkeys were given the deficient diet supplemented with 1 mg. of riboflavin daily, and these presented the characteristic signs and died in periods of time similar to the periods of survival of monkeys receiving the deficient diet alone. Nicotinic acid, either alone or in combination with riboflavin and thiamin chloride, failed to alter appreciably the course of the deficiency manifestations. Thus, it is evident that this nutritional cytopenia is not the result of a deficiency of vitamin B, riboflavin or nicotinic acid. The deficient diet supplemented with either 10 Gm. of dried brewers' yeast or 2 Gm. of liver extract (Cohn fraction G) daily supported good growth, permitted normal body development and maintained a normal blood picture over long periods. It is obvious that yeast and liver extract contain a substance essential to the nutrition of the monkey which is not identical with any of those factors of the vitamin B complex that have been chemically identified. The authors have proposed the term "vitamin M" for this factor which prevents nutritional cytopenia in the monkey. FROM AUTHORS' SUMMARY.

CORNEAL VASCULARIZATION IN RIBOFLAVIN LACK. O. A. BESSEY and S. B. WOLBACH, J. Exper. Med. **69**:1, 1939.

Vascularization of the cornea of the rat in the absence of antecedent pathologic damage is probably a specific, and the most reliable, criterion of riboflavin deficiency. Its initiation and repair may be used for testing the biologic activity of compounds structurally related to riboflavin. The fact that the invading capillaries are easily visible in the living animal and the fact that the growth and regression of the blood vessels are under dietary control and for a considerable period of time are unaccompanied by other pathologic reactions make this method very suitable for the study of problems related to capillary growth. Bessey and Wolbach believe that the best hypothesis in explanation is that the vascularization is a response to asphyxia of the tunica propria. FROM AUTHORS' SUMMARY.

A STUDY OF BONE REGENERATION. GUSTAV LEVANDER, Surg., Gynec. & Obst. **67**:705, 1938.

The specific osteoblastic theory holds that regeneration of bone may take place from preexisting cells belonging to the different layers; the metaplastic theory maintains that bone may be formed as a result of a process of transformation of nonspecific connective tissue. An essential part of the first theory concerns the predominant role of periosteum. Experimental evidence, however, has shown that it is only the periosteum of the growing skeleton that has the power to stimulate the formation of bone. Also it is known that when fully differentiated hard bone tissue is grafted, new bone is always formed. In a series of rabbits Levander first studied the mode of origin of new bone after transplanting into soft parts hard bone tissue stripped of periosteum. New bone formed from the mesenchymal tissue in the areas surrounding the graft. In his opinion, the specific stimulus necessary for the formation of new bone is brought to the mesenchyme in the form of a substance liberated from the graft and conveyed with the lymph into the surrounding areas. In a second series of experiments alcoholic extracts of bone tissue were injected into soft tissues of the abdominal wall as far as possible from any preexisting bone. Cartilage or bone was formed at the seat of injection in 22 per cent of the rabbits. Alcohol alone or alcoholic extracts of other tissues gave negative results in a control series. On the basis of these experiments it is felt that bone regeneration takes place as a result of activation of the nonspecific mesenchymal tissue by some specific bone-forming substance. This supports the heteroplastic theory of bone formation and conforms with the views of Spemann with regard to embryonic development. WARREN C. HUNTER.

Pathologic Anatomy

CORONARY ARTERY DISEASE ANALYZED POST MORTEM. W. H. GORDON, E. F. BLAND and P. D. WHITE, Am. Heart J. **17**:10, 1939.

In a study of the records of 3,400 consecutive postmortem examinations made at the Massachusetts General Hospital between February 1925 and September 1937 the following facts were ascertained: After the age of 70 years there was no difference in the incidence of disease of the coronary arteries in men and women. Before that age there was much more of this disease in men than in women. The incidence and the degree of coronary atherosclerosis were found to be significantly greater in the 600 patients from the private departments than in the 2,800 patients from the general wards. The greatest difference occurred in the middle-aged series in which coronary occlusion was found to be twice as frequent in the private patients. This difference was most striking in middle-aged men. These observations are in general agreement with clinical impressions.

FROM AUTHORS' SUMMARY.

PHARYNGEAL PITUITARY GLAND. R. H. MELCHIONNA and R. A. MOORE, Am. J. Path. **14**:763, 1938.

A small mass of typical or atypical pituitary tissue was found in the pharyngeal mucosa in 51 of 54 unselected cases examined. The differentiated cells in the pharyngeal pituitary gland are histologically identical with those in the anterior lobe of the pituitary gland, but there are relatively few chromophilic cells. Under normal conditions of growth and activity it is unlikely that the pharyngeal pituitary gland has any significant physiologic function, but in some cases of altered structure or activity of the pituitary gland it cannot be denied that the pharyngeal pituitary gland may undergo structural alterations and serve as an endocrine organ.

FROM AUTHORS' SUMMARY.

GIANT INTERSTITIAL CELLS OF THE TESTIS. A. A. NELSON, Am. J. Path. **14**:831, 1938.

In a study of microscopic sections of 721 testes from a series of 470 autopsies on men 18 years of age or more, giant interstitial (Leydig) cells having from 4 to 30 (usually 8 or 10) nuclei were found in 85 testes. They were found at all ages and in all sorts of general disease conditions. They do not appear to have been described previously, although their existence has been hinted at. The observations of Berger and others on the "sympathicotropic" or "hilus" cells have been confirmed and extended. It is generally agreed that these cells in the testis are identical with the ordinary interstitial or Leydig cells. The author proposes the name "extraparenchymal interstitial cells" or "extraparenchymal Leydig cells" as best describing them.

FROM AUTHOR'S SUMMARY.

PULSATING ANGIOMA (GENERALIZED TELANGIECTASIA) OF THE SKIN ASSOCIATED WITH HEPATIC DISEASE. D. H. WILLIAMS and A. M. SNELL, Arch. Int. Med. **62**:872, 1938.

The association of cutaneous pulsating angioma (generalized telangiectasia) and hepatic disease is not uncommon. The association probably depends on two inherent developmental characteristics which are closely related. The cutaneous lesions are primarily angiomatous and have a border of radiating telangiectatic vessels. These lesions, the development of which represents a recessive characteristic, are identical with those of hereditary hemorrhagic telangiectasia, the development of which represents a dominant characteristic. The hepatic disease is usually chronic, with cirrhosis and atrophy of the hepatic parenchyma predominating. Alcohol, arsenic and syphilis are associated factors.

ARTERIOLES OF THE PANCREAS, LIVER, GASTROINTESTINAL TRACT AND SPLEEN IN HYPERTENSION. C. G. MORLOCK, Arch. Int. Med. **63**:100, 1939.

No measurable changes in the arterioles due to age were found in the pancreas, liver, gastroenteric tract or spleen in normal persons. Measurable thickening of the arterioles, with reduction of the ratio of the thickness of the wall to the diameter of the lumen, was found in hypertensive patients of all ages. In severer hypertension there was greater thickening of the walls, with greater reduction of the ratio. Histologically, in the early stages the change was hyperplasia of the media, and in the later stages, degeneration and fibrosis. In the more severely damaged vessels degeneration of the intima was prominent, the latter composing the greatest portion of the wall. Arterioles of equal caliber showed varying degrees of pathologic change. From a histologic standpoint the pancreas exhibited the most pronounced arteriolar changes, but as a tissue the pancreas suffered no more than the other organs. The clinical classification of hypertension on the basis of severity of symptoms is substantiated by the corresponding degree of pathologic change in the arterioles. No attempt is made to establish a causal relationship between hypertension and the arteriolar changes found with hypertension.

SABRO TASHIRO.

THE MEDULLOBLAST AND THE MEDULLOBLASTOMA. J. KERSHMAN, Arch. Neurol. & Psychiat. **40**:937, 1938.

From studies of embryos and fetuses by means of Hortega's silver method Kershman concluded that medulloblasts—the composite elements of medulloblastoma, considered hypothetic—actually exist. Medulloblasts occur only in the cerebellum, where they are identified with the cells of its external granular zone. These cells are bipotential, for they give rise to neuroblasts and spongioblasts. The latter are considered by Kershman medulloblasts which do not occur in any other part of the central nervous system—for instance, the spinal cord or the cerebral hemispheres—and for this reason give rise to medulloblastoma only in the cerebellum.

G. B. HASSIN.

PERINEURIAL CYSTS OF THE SPINAL NERVE ROOTS. I. M. TARLOV, Arch. Neurol. & Psychiat. **40**:1067, 1938.

Tarlov described 5 cases of cysts of the sacral and coccygeal roots of the spinal cord. The age of the patients ranged from 49 to 63. The cysts varied in diameter from that of a pinhead to 2 cm. and originated in the perineural spaces, which, according to Tarlov, are bordered by the arachnoid and pia. The walls of the cysts, which were continuous with the dural and arachnoid membranes, consisted of several layers of arachnoid cells with a delicate connective tissue framework, while the inner border was formed of flattened endothelial cells and endoneurial reticulin. Tarlov thinks that such cysts may account for the root pains of sciatica in some cases and that they probably have their origin in an inflammatory process which seals off a portion of the perineurial space.

G. B. HASSIN.

GENESIS OF MICROGLIA IN THE HUMAN BRAIN. J. KERSHMAN, Arch. Neurol. & Psychiat. **41**:24, 1939.

The microglia which is considered by some ectodermal, by others mesodermal, has been studied by Kershman with the silver method of Hortega in human embryos of from eight to twenty-nine weeks. He concluded that microglia is a mesodermal element and originates from certain areas located underneath the meninges, in connection with inferior choroid plexus around blood vessels; in short, wherever the cerebral and mesodermal tissues are in near proximity. The various stages of growth of a typical microglial cell were traced. Where youngest it appears in an ameboid form, which is gradually transformed into a typical microglia cell. The earliest appearance of microglia seems to occur simultaneously

with that of the intracerebral blood vessels. The precursor of the microglial elements are wandering mesodermal elements, the histiocytes of mesodermal tissues. In short, microglia cells are derived from embryonic mesenchymal cells.

G. B. HASSIN.

THE HEMATOENCEPHALIC BARRIER. L. S. KING, Arch. Neurol. & Psychiat. **41**:51, 1939.

Trypan blue injected intravenously into animals colors the dura, the stroma of the choroid plexus and the parenchyma of every organ in the body except the brain. Here only the anterior half of the pituitary body, the pineal body and the area postrema become blue. On the other hand, dye injected into the subarachnoid space causes marked coloring of the cerebral parenchyma. King attempted to explain only the effects of intravenous injections. He holds that trypan blue is attracted only by organs that are relatively rich in connective tissue (the dura or the choroid plexus, for instance). In other words, the affinity of the connective tissue for trypan blue is the cause of this phenomenon and not the so-called hematoencephalic barrier which is supposed to be put up by the endothelium and to prevent the dye from passing into the parenchyma. There is increased affinity for trypan blue in inflammatory conditions of the brain. With long-continued administration of trypan blue many cells within the parenchyma stain vitally, especially around the central canal and cornu ammonis, where the dye is flocculated by "suitable" cells.

G. B. HASSIN.

EARLY PHASES OF PROSTATIC HYPERPLASIA. C. L. DEMING and C. NEUMANN, Surg., Gynec. & Obst. **68**:155, 1939.

The material for the study of the early lesions of prostatic hyperplasia was obtained at autopsies on subjects of 45 years and upward. Whole sections of glands were stained with hematoxylin-eosin and Masson's trichrome stain, the latter to differentiate muscle from fibrous tissue. Glands containing minute, early lesions were sectioned serially. The first change in benign enlargement of the prostate is primarily a multiplication of fibromuscular elements, resembling the early stages of uterine fibromyoma. The posterior part of the urethra and the internal vesical sphincter contain muscle fibers which are derived from the same embryologic source as those of the uterus. The glandular portion of the tumor is derived from ducts adjacent to the fibromyomatous nodule and not from glands. The suburethral glands of Albarran are not involved in the early stages. It is probable that the solid nodule produces some stimulating effect on the epithelium of the duct wall, causing its epithelium to bud and invade the solid nodule and form glands within it. The fibromyomatous tissue is overgrown by a more rapidly growing duct and glandular tissue, with the result that the nodule in its later stages acquires the appearance of an encapsulated glandular tumor.

WARREN C. HUNTER.

RUPTURE OF ANEURYSM INTO PITUITARY ADAMANTINOMA. H. BUESS, Beitr. z. path. Anat. u. z. allg. Path. **101**:335, 1938.

Buess reports a unique case of fatal rupture of an aneurysm of the right anterior cerebral artery into an intrasellar cystic pituitary adamantinoma. The clinical and pathologic manifestations are discussed.

R. J. LEBOWICH.

PANCREAS, HYPOPHYSIS AND THYROID IN CHILDREN OF DIABETIC MOTHERS. H. OKKELS and E. BRANDSTRUP, Acta path. et microbiol. Scandinav. **15**:268, 1938.

The pancreatic islets in children of diabetic mothers are larger and more numerous than is normal. Accompanying the insular hypertrophy there is an

increase in the differentiated epithelium of the anterior lobe of the pituitary. The thyroid gland also shows signs of increased activity. All these alterations have a common cause, probably the hyperglycemic condition induced in the fetus. This condition possibly effects the changes observed in the endocrine glands by the intermedium of the central nervous system. FROM AUTHORS' SUMMARY.

Microbiology and Parasitology

MURINE AND HUMAN LEPROSY. A. W. SELLARDS and H. PINKERTON, Am. J. Path. **14**:421 and 435, 1938.

Inoculation of murine leprosy into the brains of monkeys (Macacus rhesus), rabbits, white rats, ordinary white mice and guinea pigs resulted in the development of progressive lesions in all except the guinea pigs. White mice of mixed breeds were also readily infected by inoculation in the spleen, the liver or the peritoneal cavity, but on subcutaneous inoculation they usually showed only an abortive infection. Mice of some inbred strains frequently acquired well marked local lesions on subcutaneous inoculation, and subsequently showed extensive metastases. Murine leprosy has been maintained by serial passage in white mice of mixed breeds for four years. Various routes of inoculation have been used. Massive infections have developed, providing excellent material for experimental purposes. The bacillus of rat leprosy produced infection in the murine rodents, white rats and white mice, according to the manner of a micro-organism developing in a favorable host, but in rabbits and monkeys the circumstances of its multiplication were apparently less favorable. Monkeys (M. rhesus) inoculated with human lepromatous material were kept under observation for more than one year. Lepra bacilli were seen in small numbers in smears of inguinal glands. Transfers of the infection were made to other monkeys, in which similar low grade infections developed during a period of more than a year. Portions of the pia mater of one of these monkeys were implanted intracerebrally in white rats; fifteen months later many acid-fast bacilli were found in the brain of 1 rat. Briefly, human lepra bacilli were maintained in animals for a period of three and three-fourths years, but no progressive disease and no active lesions developed.

The lepra cells in murine leprosy are derived largely from mesenchymal cells belonging to the reticuloendothelial system. Exceptionally, however, epithelial cells, specifically those of the epidermis, testicular tubules and epididymis, became distended with lepra bacilli. This observation suggests that the relative resistance of epithelial cells to infection may depend on the inability of lepra bacilli to enter them rather than on intracellular conditions unfavorable to their growth. In infected rats and mice surviving for a long time the tissues of practically all the organs were extensively replaced by nonvacuolated lepra cells distended with bacilli, but renal tissue contained relatively few of these cells. In the progressive local and metastatic lesions produced with Bacillus leprae murium in rabbits and monkeys the lepra cells were often vacuolated, and acid-fast bacilli were much less numerous in the lesions in these animals than in those produced in rats and mice, and were found only after prolonged search. Nonpathogenic acid-fast bacilli injected intracerebrally were taken up by macrophages and neutrophils but disappeared from the lesions in a few weeks, never producing metastatic lesions. Virulent tubercle bacilli, although innocuous in mice when injected subcutaneously, produced progressive and metastatic infection when injected intracerebrally. The lesions were noncaseating, and the tubercle bacilli were found largely within macrophages, so that these lesions closely resembled those of leprosy.

FROM AUTHORS' SUMMARIES.

RELATION OF HUMAN ENCEPHALITIS TO ENCEPHALOMYELITIS IN HORSES. C. M. EKLUND and A. BLUMSTEIN, J. A. M. A. **111**:1734, 1938.

In the summer of 1937 encephalitis occurred in 6 farmers in localities where equine encephalomyelitis was prevalent. The blood serum of 1 of 3 patients whose serums were tested was shown to neutralize the western strain of equine encephalomyelitis. As far as is known, this is the first time that neutralization of the virus of equine encephalomyelitis by human serum has been found.

FROM AUTHORS' SUMMARY.

HUMAN ENCEPHALITIS: EIGHT FATAL CASES, WITH FOUR IN WHICH THE DISEASE WAS DUE TO THE VIRUS OF EQUINE ENCEPHALOMYELITIS. C. WESSELHOEFT, E. C. SMITH and C. F. BRANCH, J. A. M. A. **111**:1735, 1938.

Fatal acute encephalitis was observed in 8 patients. All the patients resided in an area where an epidemic of the eastern strain of equine encephalomyelitis involved more than 200 horses during the same period and where mosquitoes were unusually prevalent. Autopsies were performed on 7 of these 8 patients, and the virus of the eastern strain of equine encephalomyelitis was recovered from the brains of 4. The virus of the eastern strain of equine encephalomyelitis causes in human beings a profound acute disseminate and focal encephalomyelitis, characterized by intense vascular engorgement, perivascular and parenchymatous cellular infiltration and extreme degenerative changes in the nerve cells. The gross pathologic manifestations are not specific. When the infection is severe, the microscopic reaction can readily be distinguished from any of the common types of encephalitis.

FROM AUTHORS' SUMMARY.

PYOGENIC FILTERABLE AGENT IN THE ALBINO RAT. W. H. WOGLOM and J. WARREN, J. Exper. Med. **68**:513, 1938.

A filtrable agent resembling virus is described. It was encountered in sarcoma 39, a propagable neoplasm of the white rat, and has now been maintained in this species for twenty-eight passages over a period of some seven months without appreciable loss in virulence. Its chief effect is the production of large abscesses in an animal species comparatively resistant both to viral diseases and to suppuration. The white mouse is more susceptible than the white rat, the rabbit less so, and the guinea pig is highly resistant. The agent was repeatedly recovered from sarcoma 39 when this was treated in special ways, but under the ordinary circumstances of routine transplantation it did not manifest itself. As yet there is no certainty as to where it came from or how it maintains itself under natural conditions.

FROM AUTHORS' SUMMARY.

ULTRACENTRIFUGATION OF VACCINE VIRUS. E. G. PICKELS and J. E. SMADEL, J. Exper. Med. **68**:583, 1938.

Ultracentrifugal studies on the CL dermal strain of vaccine virus indicate the following characteristics of the elementary bodies: A stable suspension of Paschen bodies in a dilute buffer solution of p_H 6.2 to 8 sediments with the formation of a characteristic primary boundary which consistently shows a spread of approximately 14 per cent. The principal sedimentation boundary is accompanied frequently by one or several more rapidly moving boundaries which probably are produced by groups of agglutinated elementary bodies consisting of two or more particles. Occasionally the principal boundary may exhibit an irregular or peculiar behavior, a fact which necessitates a careful selection of material and the performance of many experiments for accurate interpretation of results. The sedimentation constant of the slowest moving particles forming the principal boundary is computed to be 49.1×10^{-11} cm./seconds/dyne. On the basis of this sedimentation rate the average diameter of the smallest virus particles in appre-

ciable amounts is estimated at 236 microns. If the boundary spread is due principally to slight differences in particle size, then the largest single elementary bodies are approximately 252 microns in diameter.

FROM AUTHORS' SUMMARY.

CORRELATION BETWEEN THE GROUP SPECIFIC SUBSTANCES AND BIOCHEMICAL CHARACTERISTICS OF HEMOLYTIC STREPTOCOCCI. M. C. HECHLER and M. A. FARRELL, J. Infect. Dis. **63**:225, 1938.

"A study of the serological and biochemical properties of 42 strains of hemolytic streptococci led to the following conclusions:

"By means of the precipitin test 37 of the 42 strains are classified into the 7 serological groups of Lancefield.

"Five of the 7 serological groups, in a large majority of cases, show a definite relation to the source of the strains.

"The biochemical and cultural characteristics of all strains within a group are somewhat variable although predominant group reactions are noted. Certain correlations are indicated between the group specific substances and fermentative reactions."

AUTHORS' SUMMARY.

TRICHINOSIS IN CLEVELAND: POSTMORTEM EXAMINATION OF DIAPHRAGMS AND SKELETAL MUSCLES FROM 100 CONSECUTIVE AUTOPSIES. C. H. EVANS JR., J. Infect. Dis. **63**:337, 1938.

"An incidence of 36% of trichinosis for the Cleveland area is indicated by postmortem examination of diaphragms and skeletal muscles from 100 consecutive autopsies.

"In this series the usual procedure of examination of diaphragm by the digestion-Baermann method alone revealed an incidence of 20%.

"The application of the compression-microscopic and the digestion-Baermann methods shows variations in the number of organisms recovered, but together they give a more nearly true result than with either alone.

"The examination of skeletal muscles such as the intercostals and the sternomastoid in addition to the diaphragm gives a higher incidence of infestation than is revealed by examination of the diaphragm alone."

AUTHOR'S SUMMARY.

VIABILITY OF PNEUMOCOCCI IN DRIED SPUTUM. E. G. STILLMAN, J. Infect. Dis. **63**:340, 1938.

Pneumococci have been recovered from dried sputum which has been exposed to diffuse daylight at room temperature for months.

Pneumococci will live in dried sputum stored in the cold, on an average, four or five times longer.

FROM AUTHOR'S SUMMARY.

EPIDEMIC INFLUENZA VIRUS. W. SMITH and C. H. ANDREWES, Brit. J. Exper. Path. **19**:293, 1938.

An antigenic analysis of 28 strains of influenza virus isolated from human patients has been made by means of cross neutralization tests in mice. Many antigenic components enter into the constitution of the various strains. Of these components, four appear to possess major significance; they were found in very different proportions in the different strains. On the basis of their content of the four major antigens it was possible to classify the strains into three main categories, namely, highly specific strains, relatively nonspecific strains and intermediate strains. A strain of virus can be shown to absorb from a heterologous serum only those antibodies which correspond to its own antigenic components. Active immunization experiments with mice show that the protection conferred by vaccination with a single strain is most effective against that strain. The

efficacy of a vaccine against a heterologous virus is largely dependent on the closeness of antigenic relation between the strains concerned. Experiments with ferrets suggest that in animals with some basic immunity the response to vaccination may be less specific than that in animals with no previous experience of influenza. The difficulties of applying the results of animal experiments to human vaccination are discussed. FROM AUTHORS' SUMMARY.

MICROSCOPIC OBSERVATIONS ON THE BACTERIOPHAGE OF BACTERIUM COLI. K. B. MERLING-EISENBERG, Brit. J. Exper. Path. **19**:338, 1938.

The lytic action of the bacteriophage of Bacterium coli has been observed and photographed in the following stages: Particles appear within the bacterial cell, which swells up and finally bursts, discharging the particles into the surrounding medium. The bacteriophage is distinguished from nonspecific particles, and its size is found to be 25 millimicrons. FROM AUTHOR'S SUMMARY.

THE VIRUS OF TRACHOMA. A. CUÉNOD and ROGER NATAF, Arch. Inst. Pasteur de Tunis **27**:284, 1938.

These studies confirm the authors' preceding studies of the presence of rickettsia-like bodies in trachoma. Cultures in series on the chorioallantoic membrane of the egg show lesions on the membrane, virulence for the embryo and frequent presence of rickettsioid bodies. This cultivation permits new analogies to be found between the etiology of trachoma and that of classic and murine typhus and permits the formulation of the hypothesis that epithelial inclusions of the Prowazek and Halberstaedter types and rickettsias and rickettsioid bodies are different stages in the development of the same virus. The studies suggest also the use of potassium cyanide in the treatment and prophylaxis of trachoma and of various infections due to rickettsias. FROM AUTHORS' SUMMARY.

Immunology

FATAL ANAPHYLACTIC SHOCK IN MAN. J. ZISKIND and H. J. SCHATTENBERG, Arch. Int. Med. **62**:813, 1938.

Fatal anaphylactic shock followed a second intravenous injection of a foreign protein. At necropsy marked congestion of the liver and more moderate congestion of the other organs were noted. General enlargement of the lymph nodes and persistence of the thymus gland were also noted. The significant microscopic changes were as follows: The hepatic sinusoids were dilated and congested, as were also the alveolar capillaries of the lungs. Within these vessels the leukocytes and the eosinophils were apparently relatively increased. The alveoli in some areas were compressed; those in others, dilated. In some instances their walls were ruptured. In the spleen the malpighian corpuscles were increased in size and had large germinal centers. It is suggested that the mechanism of death was of the so-called dog type, namely, capillary dilation, especially in the liver, with corresponding fall in blood pressure and cardiac failure.

FROM AUTHORS' SUMMARY.

PREVENTION OF HYPERSENSITIVITY IN TUBERCULOSIS. R. H. FOLLIS JR., Bull. Johns Hopkins Hosp. **63**:283, 1938.

Animals infected with the tubercle bacillus and prevented from becoming hypersensitive by treatment with old tuberculin show less caseation in their lesions than do control animals in which hypersensitivity has developed. In some of the nonhypersensitive animals pulmonary lesions develop which are marked by extraordinary proliferation of the acid-fast bacilli. Since these lesions did not develop

in the majority of the nonhypersensitive animals and since the same lesions were found in some of the animals of the control groups, shown to be hypersensitive, it is concluded that lack of hypersensitivity does not play a role in producing lesions of this type. FROM AUTHOR'S SUMMARY.

POLYSACCHARIDES FROM TUBERCLE BACILLI. F. R. SABIN, A. L. JOYNER and K. C. SMITHBURN, J. Exper. Med. **68**:563, 1938.

Purified tuberculopolysaccharides are relatively innocuous both to normal and to tuberculous guinea pigs. Both tuberculopolysaccharide and polysaccharides from pneumococci call larger numbers of leukocytes from the blood vessels than do salt solution and dextrose and trehalose. The mechanisms controlling the delivery of lymphocytes and neutrophils into the blood stream are different. Slight irritation of the peritoneal lining slows the delivery of lymphocytes to the blood stream. There are two phases in the reaction of the bone marrow to intraperitoneal injections. Correlated with the draining of neutrophils from vessels to tissues, owing to the presence of foreign materials in the latter, there is a draining of young neutrophils from the marrow into the sinuses of the marrow as these same materials reach the sinuses. The subsequent disintegration of the neutrophils extravasated into the tissues is correlated with increased myeloid activity in the marrow. FROM 'AUTHORS' SUMMARY.

REACTIONS TO TUBERCULOPROTEINS AND TUBERCULOPHOSPHATIDE. K. C. SMITHBURN and F. R. SABIN, J. Exper. Med. **68**:641, 1938.

Prior observations on the cellular reactions to tuberculophosphatide are confirmed and the reactions compared with reactions induced by this material in tuberculous animals. In the latter the response is accelerated and augmented and simulates the Koch phenomenon. Tuberculoprotein produces no macroscopic reaction in normal animals. The microscopic reaction of neutrophils and monocytes regresses in less than a week. The same material in tuberculous animals causes a response characterized by more or less hemorrhage and necrosis, tissue degeneration and infiltration by neutrophils and monocytes. Late in the reaction there may be a few epithelioid cells and foreign body giant cells. Preparations of tuberculophosphatide which contain no tubercle bacilli or only a few induce the typical cellular response but do not induce hypersensitiveness to tuberculin. Repeated intradermal skin test injections of tuberculoprotein MA-100 in normal guinea pigs may be followed by mild hypersensitiveness to subsequent injections. FROM AUTHORS' SUMMARY.

RESPONSE OF IMMUNIZED MICE TO REINOCULATION WITH THE VIRUS OF ST. LOUIS ENCEPHALITIS. E. A. COOK, J. Infect. Dis. **63**:206, 1938.

If seven to twenty-one days after mice have been given one or two injections of the virus of St. Louis encephalitis by either the subcutaneous or the intraperitoneal route they are reinoculated intracerebrally or intranasally, they may show sudden flaccid paralysis of the limbs, with a fatal or a nonfatal outcome. Similar results may be observed on intracerebral inoculation of mice after one or two previous intranasal inoculations. The incubation period of the paralytic condition is five to fourteen days, being shorter (five to eleven days) after intracerebral than after intranasal injection (eleven to fourteen days).

Suspensions of the brains or cords of such paralyzed animals when injected into normal mice give rise to typical encephalitis. Virus exists usually in slightly higher relative titer in the cord than in the brain but is in comparatively low titer in both and disappears rapidly. It is not detectable in the central nervous system seven, fourteen or twenty-one days after the injection of a nonlethal dose.

The localization of the paralytic symptoms is not correlated with the site of injection.

The serum of mice given a single subcutaneous or intranasal injection of a nonlethal amount of virus possesses little or no neutralizing capacity. However, in animals so treated a certain degree of immunity exists, as shown by their resistance to subsequent inoculation with virus in amounts rapidly lethal to controls.

The chief pathologic changes in the cords of paralyzed mice are vascular congestion, perivascular and diffuse round cell infiltration, proliferation of microglia and nerve cell degeneration, especially in the anterior horns.

FROM AUTHOR'S SUMMARY.

LETHAL TOXINS OF HEMOLYTIC STREPTOCOCCI. E. W. TODD, Brit. J. Exper. Path. **19**:367, 1938.

Group A hemolytic streptococci produce two distinct varieties of streptolysin, which are both lethal to mice: (1) streptolysin O, which is sensitive to oxygen; (2) streptolysin S, which is extractable with serum. These two streptolysins are neutralized by separate, entirely unrelated antibodies. Antistreptolysin O protects mice against either death or hemoglobinuria which may be caused by streptolysin O, but it does not protect against streptolysin S. Antistreptolysin S protects mice against either death or hemoglobinuria which may be caused by streptolysin S, but it does not protect against streptolysin O. Antistreptolysin S does not protect mice against living cultures of hemolytic streptococci, and it does not enhance the protective action of antistreptolysin O. FROM AUTHOR'S SUMMARY.

ANTIRABIC IMMUNIZATION. I. J. KLIGLER and H. BERNKOPF, Brit. J. Exper. Path. **19**:378, 1938.

Experiments are reported which indicate that formaldehydized tissue cultures of rabies virus possess considerable immunizing capacity for mice if injected intraperitoneally. The immunity to an intracerebral infection is incomplete; a little less than half of the mice given five to seven injections of the vaccine possessed solid immunity to 10 lethal intracerebral mouse doses; about 42 per cent possessed no immunity. Immunity to an intraperitoneal or subcutaneous infection is more easily established. Even a single intraperitoneal treatment with the inactivated culture virus is sufficient to protect mice against an intraperitoneal infection with a dose of fresh virus which kills about two thirds of the control mice. A similar immunity is conferred against a subcutaneous infection except that a larger amount of vaccine is required. Of special interest, perhaps, are the data showing that immunized mice which survive an intraperitoneal infection possess a greater degree of immunity than do untreated mice which survive such an infection. Primary treatment with inactive virus affects to a marked extent the immunity of these mice to an intracerebral infection. Mice which received diluted vaccine (1 : 10) or fewer doses possessed a lower degree of immunity than the others, although all of them survived an intraperitoneal or subcutaneous infection with fresh brain virus. Only half of the mice which survived an intraperitoneal infection without previous vaccination were immune to an intracerebral infection, whereas 84 per cent of previously immunized mice proved immune. A single dose of a rabies virus culture which had a very low virulence for mice, if injected intraperitoneally, conferred on the treated mice a high degree of immunity against a subsequent intracerebral infection with test brain virus. FROM AUTHORS' SUMMARY.

Tumors

LUNG TUMOR DEVELOPMENT IN A RESISTANT STRAIN OF MICE. M. G. SEELIG and E. L. BENIGNUS, Am. J. Cancer **34**:391, 1938.

A 10 per cent mixture of carcinogenic tar in lampblack failed to induce cancer of the lung in any of 100 genetically tumor-resistant mice which inhaled the mixture over a period of ten months and which at autopsy showed a heavy deposition

of the lampblack in the lungs. This result is in marked contrast to that of a previous experiment in which with chimney soot there was induced an 8 per cent incident of pulmonary tumors in a less resistant strain of mice. A 4 per cent spontaneous incidence was observed in a control group of 50 animals in spite of the fact that this strain when living under a Bar Harbor environment showed less than 1 per cent incidence of tumor of the lung. It is entirely possible that the difference of tumor incidence in this and in our earlier experiment is due to (*a*) the varying tumor productivity of the particles used in the first experiment as contrasted with the softer, less traumatizing particles of lampblack used in the second experiment. Because pulmonary growths in mice may be so small as to escape macroscopic detection, it is believed that serial sectioning and microscopic examination of tissue are essential in determining the incidence of tumor of the lung. Unfortunately, it seems to be true that environmental conditions alter the genetic reaction to tumor growth on the part of even pure strain animals. Equally unfortunate is the lack of agreement of opinion, or at least of certainty, regarding the histology of pulmonary tumors in mice. FROM AUTHORS' SUMMARY.

TRANSPLANTABLE LYMPHOSARCOMA IN MICE. M. R. LEWIS, Am. J. Cancer **34**: 399, 1938.

Lymphosarcoma developed in 3 mice that were given injections of 0.8 mg. of 1,2,5,6-dibenzanthracene dissolved in 0.4 cc. of sterile olive oil. Tumors (Carnegie 172, 226 and 233) arose at the sites of injection seventy-five, one hundred and fifteen and two hundred and thirty-seven days later, respectively. They were composed of malignant lymphoctes. Two of them arose from the axillary lymph nodes and the third from the thymus. One of them (no. 172) was transplanted through twelve generations in mice of the strain in which it originated. It was found to be 100 per cent transplantable in mice of the same strain but not transplantable in mice of other strains. The tumor was passed by implantation of whole blood or pieces of lung, liver, spleen, kidney, lymph node or tumor but not by implantation of blood plasma or of the supernatant fluid of centrifuged extracts of the tumor. FROM AUTHOR'S SUMMARY.

FREEING WALKER TUMOR FROM CONTAMINATING BACTERIA. R. E. GARDNER and R. R. HYDE, Am. J. Cancer **34**:442, 1938.

Metastases are readily obtained by inoculating small pieces of Walker tumor no. 256 into the tails of young susceptible rats. When the implanted tumor is contaminated with bacteria the metastases usually prove to be sterile, especially those from the region of the retroperitoneal lymph node.

FROM AUTHORS' SUMMARY.

MALIGNANT GIANT CELL TUMOR OF BONE. F. W. STEWART, B. L. COLEY and J. H. FARROW, Am. J. Path. **14**:515, 1938.

The authors have been unable to arrive at a satisfactory descriptive term for the malignant giant cell tumors of bone. They believe that it is perhaps best to retain the designation "malignant giant cell tumor," since it carries at least a definite connotation. They state that the efforts to establish hard and fast lines of distinction for cells involved in bone development have made the description of the histogenesis of bone most complex. Through the ultracytologic analyses of various histologists, according to the authors, cells have acquired individualities which they probably do not merit or merit only in a transient sense. The cell elements of giant cell tumors cannot be separated from the connective tissue cells and vessels which are involved in the histogenesis of bone and which evolve in different directions, dependent on the physicochemical conditions of the period. The authors are much in sympathy with the views of Moschcowitz as expressed in his paper on the relation of angiopenesis to ossifica-

tion, and they see many similarities in the development of malignant giant cell tumors. They doubt that one can specifically state that a giant cell tumor is a tumor of giant cells, intervening connective tissue cells or angioblastic elements or that the malignant giant cell tumor is a sarcoma of giant cells or of angioblastic elements or an endothelioma or a granulation tissue sarcoma, since there is great difficulty in separating the elements of the tumor into permanent entities. In their own cases they observed no true bone formation, and yet it would not surprise them if a tumor with this evolutionary pattern should appear. Despite the tendency, which they also have followed, to reject as giant cell tumors of malignant type those tumors in the metastases of which cartilage has appeared, they think the rejection may not be necessarily warranted.

Thus the form assumed by the process known as giant cell tumor will be found to depend on the nature of the circumstances, physical and chemical, which have initiated the process plus the extrinsic factors that have interfered with its normal evolution. Until the giant cell tumor and its malignant evolution are better understood, the interpretation of this type of tumor must remain in a speculative phase. FROM AUTHORS' SUMMARY.

CARCINOMA IN THE LEOPARD FROG. B. LUCKÉ, J. Exper. Med. **68**:457, 1938.

An epithelial tumor with acidophilic intranuclear inclusions frequently occurs in the kidneys of leopard frogs. This tumor usually has the appearance of an infiltrating and destructive adenocarcinoma. When large, it not uncommonly metastasizes; less often it is more orderly and adenomatous. When inoculated as living fragments or as a cell suspension into the lymph sacs, cranial cavity or abdomen, it gives no significant local growths, and the implanted material is resorbed. However, in approximately 20 per cent of the frogs surviving inoculation for more than six months, renal tumors develop which are like the "spontaneous" neoplasms. The incidence far exceeds that in the controls. Desiccated and glycerinated tumor injected into the abdomen gives the same result as living tumor; in somewhat over 20 per cent of animals surviving more than six months renal tumors occur. In alien species of frogs, no such tumors are produced by inoculation of either living or desiccated tumor. These experiments indicate the probability that the tumor of the kidney of the leopard frog is caused by an inclusion-forming, organ-specific virus. FROM AUTHOR'S SUMMARY.

PRIMARY RETICULUM CELL SARCOMA OF BONE. F. PARKER JR. and H. JACKSON JR., Surg., Gynec. & Obst. **68**:45, 1939.

The type of primary tumor of bone described apparently constitutes a definite group, hitherto unrecognized and unprovided for in the classification of the Bone Sarcoma Registry. Although such a tumor was suggested as a possibility in 1931, the first positive diagnosis of it was not made until 1936. Prior to this tumors of this type had been variously classified as Ewing's sarcoma, Hodgkin's disease, lymphosarcoma, osteogenic sarcoma, leukosarcoma or inflammation. Because of the more serious lesions with which it may be compared, the characteristics and clinical course in 17 cases, in 10 of which it involved males, are described. Primary reticulum cell sarcoma of bone differs materially from the generalized form in that in the great bulk of cases it occurs in persons under the age of 40 and involves the long or flat bones instead of the vertebrae and skull. Despite local extensive involvement and roentgenographic evidences of a massive and destructive lesion, the patient remains in good condition.

Histologically, the neoplastic cells have round, oval, indented or lobulated nuclei, twice the size of a lymphocyte, scattered chromatin granules and a considerable amount of cytoplasm. Despite the apparently malignant nature of the tumor it is quite amenable to treatment, as proved by the fact that 13 of the 17 patients are alive from six months to fourteen years after the first appearance of symptoms. Early diagnosis by biopsy followed by immediate amputation and irradiation seems to be the proper procedure. WARREN C. HUNTER.

BONE SARCOMA: FACTORS INFLUENCING THE PROGNOSIS. C. C. SIMMONS, Surg., Gynec. & Obst. **68**:67, 1939.

This report is based on the results of treatment of 47 primary malignant tumors of the long bones, excluding plasma cell myeloma, at the Massachusetts General Hospital during the period between 1920 and 1932. The prognosis is not as poor as is generally believed if the tumors are removed by radical surgical operation; it depends more on the amount of differentiation of the cells comprising the major portion of the tumor than on any one other factor. Tumors composed largely of adult fibrous tissue or cartilage offer a better prognosis than those in which the cells show marked anaplasia. Of 28 patients with osteogenic sarcoma on whom amputation was done, 39 per cent are living and without evidence of disease five or more years after operation. All patients (5) in whose tumors fibrous tissue predominated and on whom amputation was done are well; the percentage for those whose tumors showed a predominance of cartilage and who were treated in the same manner is 70; only 5.5 per cent of those with tumors made up of anaplastic cells are alive after five years. All patients with Ewing's sarcoma, whether treated by amputation or by irradiation, died of the disease.

WARREN C. HUNTER.

INFLAMMATION AND CARCINOGENESIS. S. BECK, Brit. J. Exper. Path. **19**:319, 1938.

Mice were given injections of 3,4-benzpyrene dissolved in a 30 per cent solution of turpentine in olive oil. In each instance a sterile abscess was thus induced around the benzpyrene. In other groups benzpyrene was injected into or near such abscesses. Two groups given injections of benzpyrene in the same solvents but without turpentine served as controls. In another control group abscesses were induced but no benzpyrene was injected. All groups receiving injections of benzpyrene acquired tumors and showed the fluorescence of persistent benzpyrene in the tissues for about six months. The following conclusions are drawn: Acute inflammation had no apparent influence on the rate of absorption or on the carcinogenicity of 3,4-benzpyrene in fatty solvents; the inflammatory fibrous tissue did not accelerate or retard the development of sarcoma; sarcoma did not originate from the fibrous tissue of subacute inflammation; 3,4-benzpyrene did not act at the locus of the injection but on tissues in the immediate neighborhood, through which it passed on its way to absorption. It is concluded that 3,4-benzpyrene titrates itself against the tissues, and an optimal concentration for an optimal time determines which of the susceptible cells become malignant. Probably only a few points in the tissue fulfil these conditions.

FROM AUTHOR'S SUMMARY.

THE ANTIGENIC BASIS OF TUMOR TRANSPLANTATION. P. A. GORER, J. Path. & Bact. **47**:231, 1938.

Gorer studied the behavior of a malignant tumor and leukemic tissue arising in one strain of mice when this tumor and leukemic tissue were injected into mice belonging to an unrelated pure line and also when they were injected into other members of the strain in which the tumor arose. As a rule, members of the same strain of mice were susceptible, whereas unrelated mice were resistant. In resistant mice the injection of leukemic tissue and blood gave rise to immune isoagglutinins capable of acting on the red blood cells of mice belonging to susceptible species, the isoagglutinins attaining their maximum titer seven days after the injection. Following inoculation of sarcomatous tissue the maximum titer of the isoagglutinins corresponded with the time of complete regression of the tumor, i. e., fourteen days. The highest titers were obtained after tumor grafting, and leukemic tissue gave rise to stronger isoagglutinins than blood. The titers dropped rapidly during the week following the attainment of their maxima. The serologic evidence obtained in the study agreed with the genetic evidence published in a previous paper indicating that two genes govern the transplantability of the tumor and also with the hypothesis that both genes determine isoantigenic differences.

A. S. WIENER.

HISTOLOGIC TYPES OF MENINGIOMA. J. O. W. BLAND and D. S. RUSSELL, J. Path. & Bact. **47**:291, 1938.

A series of 106 surgical specimens of dural meningioma has been analyzed. Five histologic types have been identified and described. A series of 14 tumors representative of these types has been submitted to tissue culture. In each type a great variety of cell form and growth pattern has been observed, and no cultural characteristics have been found to belong to any one histologic type of tumor. The cultural characters of meningioma have been compared with those of fetal leptomeninges and dura and adult granulation tissue. While the cultures of all the tissues studied have the features of cultures of fibroblasts, those of fetal leptomeninges differ, as a rule, from those of meningioma in not uncommonly forming epithelioid but not true epithelial sheets. This tendency toward sheet formation is considered to support the theory of the arachnoid origin of dural meningioma. A striking resemblance was found between cultures of meningioma, fetal leptomeninges and fetal dura, on one hand, and cultures of human fetal mesenchyme, on the other, both showing great polymorphism. A similar polymorphism is shown by the endothelioid cells of the leptomeninges in pathologic conditions other than neoplasm: They may form macrophages which store dyes or engulf particulate matter, and they may form fibroblasts with fibroglial fibrils. It is concluded that the leptomeninges are composed of cells which approximate to the undifferentiated mesenchymal cells with embryonic potentialities of the polyblastic system of Maximow.

FROM AUTHORS' SUMMARY.

RETICULOSIS AND RETICULOSARCOMA. A. H. T. ROBB-SMITH, J. Path. & Bact. **47**: 457, 1938.

A classification of the progressive hyperplasias and neoplasias of lymphoreticular tissue is suggested which follows Maximow's hypothesis of the pluripotency of certain primitive cells of the embryonic mesenchyme, which persist throughout life. The justification for a methodologic consideration of the lymphadenopathies has been put forward in the introduction. On such a framework a clinical analysis can be superimposed and a search made for etiologic factors. Further subdivisions may be necessary, or certain of those put forward may prove superfluous, but with classification based on cytologic aspects and structure such adjustments are possible without destroying the main concept, and it is only by uniformity of description and a scrupulous use of terms that any advance can be made.

FROM AUTHOR'S SUMMARY.

MORPHOLOGY OF HERPES VIRUS. S. NICOLAU and L. KOPCIOWSKA, Ann. Inst. Pasteur **60**:401, 1938.

In experimental herpetic encephalitis of rabbits the nuclei of the nerve cells and of the glial cells show granular masses made up of numerous elements resembling bacilli when stained with oxalated methyl blue in glycerinated diiute alcoholic acid fuchsin solution. These bodies are believed to be colonies of herpes virus. The individual elements are 0.1 to 0.2 micron in diameter. These measurements agree with those determined by ultrafiltration and ultracentrifugation.

Similar masses are found in the cytoplasm of neurons, glial cells, endothelial cells of the capillaries and monocytic cells of meningeal or parenchymatous infiltrations. After inoculation of herpes virus into the scarified cornea of the rabbit similar bodies are seen in the nuclei and cytoplasm of epithelial cells of the cornea. After inoculation of the virus into the skin of the metartarsal region of the paw intranuclear colonies are found in the malpighian cells.

In the skin of human beings suffering from herpes zoster the virus forms colonies of similar morphologic character in the nuclei of epithelial cells of the malpighian layer. After inoculation of herpes virus into a peripheral nerve many microbic cells are found between the nerve fibers, within the nerve fibers and in the axis-cylinders.

Intranuclear herpetic inclusions originate from colonies of the virus by agglutination, degeneration and fusion of the organisms. The corpuscles which result are often included in a mass of the microbic cells. They gradually become eosinophilic and tend to be separated from the surrounding cell by a halo. Cytoplasmic inclusions arise in the same way and are an expression of a resistant state of the cell.

FROM AUTHORS' CONCLUSIONS.

ROLE OF THE SKIN IN CHICKEN SARCOMA. A. BESREDKA and L. GROSS, Ann. Inst. Pasteur **60**:465, 1938.

The incubation time of Rous sarcoma in the chicken is longer after subcutaneous than after intracutaneous inoculation. When once formed, subcutaneous sarcoma progresses rapidly to a fatal issue. Intracutaneous sarcoma appears very rapidly after injection and then progresses slowly. Intracutaneous tumors may remain stationary for months. When weak doses are inoculated intracutaneously, the tumor is resorbed in about half the cases.

Chickens which have resorbed their intracutaneous tumors become refractory to subsequent subcutaneous or intramuscular inoculations. This specific, lasting and solid immunity is of a cellular nature and is not passively transferable to other animals.

FROM AUTHORS' CONCLUSIONS.

MALIGNANT TUMORS FOLLOWING PROLONGED ADMINISTRATION OF ESTROGENIC HORMONES. A. LACASSAGNE, Bull. Assoc. franç. p. l'étude du cancer **27**:96, 1938.

This is a statistical analysis of data collected during six years, concerning which several preliminary reports have been published since 1932. Selected strains of mice, some with a high and some with a low incidence of mammary adenocarcinoma in females, some known to be very rarely afflicted with spontaneous malignant tumors, were given, beginning soon after birth, weekly injections of estrogenic hormones. Mammary adenocarcinoma was observed only in strains normally subject to it, but in larger numbers and earlier than normally, and in males as well as in females. A varying number of epithelial and connective tissue tumors appeared in strains normally known to be free from such tumors. Lacassagne concludes that estrogenic hormones played a part in the production of the tumors.

I. DAVIDSOHN.

GENERALIZED LYMPHOSARCOMA OF MORE THAN NINE YEARS' DURATION. J. DUCUING, O. MILETZKY and BASSAL, Bull. Assoc. franç. p. l'étude du cancer **27**:117, 1938.

In 1928 a lymphosarcoma, the diagnosis of which was confirmed by several biopsies, was seen for the first time in a 54 year old woman who complained of edema of the right leg. A mass was present in the right iliac fossa. After that until May 1937 the patient was examined at irregular intervals, with findings of generalized enlargement of the lymph nodes in the neck, axillas, groins and mediastinum and one breast. The spleen and later the liver were markedly enlarged. The findings varied, depending on the roentgen therapy; the dosage and the dates of administration are recorded. There was moderate to marked anemia, with periods of return to normal, and leukopenia with relative lymphocytosis, but on one occasion relative lymphopenia was recorded. Marrow obtained by sternal puncture revealed a relative decrease of granulocytes and of their precursors. The response to roentgen therapy was prompt and continued through the entire thus far observed course of the disease.

I. DAVIDSOHN.

Technical

BLOOD SUBGROUPS A_1 AND A_2. I. DAVIDSOHN, J. A. M. A. **112**:713, 1939.

Clinical experience indicates (1) that the selection of a donor according to known methods does not assure absence of blood transfusion reactions; (2) that

unexpected reactions are not uncommon when a donor of the same blood group as that of the patient is employed, especially when the group is O or A, and (3) that reactions are particularly frequent when so-called universal donors are employed.

Available serologic data suggest (1) that subgroups A_1 and A_2, A_1B and A_2B are not always compatible; (2) that subgroups A_2 and A_2B are not infrequently mistaken for other groups, particularly for O and B, and (3) that some transfusion reactions, even fatal ones, are well explained by these circumstances.

The method presented offers two advantages: 1. The high-titered, easily produced and highly specific rabbit immune serum permits prompt recognition of blood group A and AB, including the feebly agglutinating subgroups A_2 and A_2B. 2. A proper dilution of the serum, as determined by titration, makes it possible to differentiate subgroup A_2 from A_1 and A_2B from A_1B without delay. Both procedures can be carried out within five minutes.

From Author's Summary.

Detection of Glutathione in the Mitochondria and Nucleus. P. Joyet-Lavergne, Compt. rend. Soc. de biol. **128**:59, 1938.

The author submits a staining method for the demonstration of glutathione in the cells of plant or animal tissues. It is based on treatment of the sections with a 1 per cent cadmium lactate, converting the glutathione into a cadmium compound optically visible under the microscope. Most of the intracellular glutathione is situated in the mitochondria and nucleus of the cells. R. J. Lebowich.

Changes Occurring in Blood Preserved During Many Weeks and the Therapeutic Effect of Such Blood. H. Gnoisky, Sang **12**:820, 1938.

Gnoisky studied the properties of preserved blood obtained from 40 dogs and 80 human beings and the effect of transfusion of such blood into 18 dogs and 6 patients. The bloods were obtained aseptically, mixed with one-fifth their volume of 6 per cent citrate solution, and placed in large sterile ampules, which were hermetically sealed after all the air had been evacuated. Portions of blood were also distributed in small ampules, which were opened at intervals for testing. All specimens were kept at 4 C. A gradual decrease in the number of red cells was observed, 15 per cent of the cells having disappeared after seventy-three days. Of the red cells surviving after ninety days, 40 per cent exhibited morphologic changes. Dog blood behaved like human blood except that the cells were more fragile. The leukocytes underwent more rapid changes than the red cells, and the lymphocytes persisted longer than the polymorphonuclears. The platelets disintegrated most rapidly, completely disappearing within a period of fifteen to twenty days. Transfusions of 150 to 280 cc. of stored blood into 6 patients gave rise in each case to a chill and a rise in temperature, but these were evanescent and not serious. This reaction was usually followed by an increase in the urobilinogen and the appearance of traces of hemoglobin in the urine. Nevertheless, in most of the cases there was a satisfactory rise in hemoglobin within a week.

A. S. Wiener.

Magnetic Electron Microscope and Its Use in Biology. F. Krause, Naturwissenschaften **25**:817, 1937.

Krause has traced the development and discussed the theoretic basis and resolving power of the Knoll-Ruska type of electron microscope. The limits of resolution are indicated. The article is illustrated with electronic photographs of cells and bacteria. The optical image sometimes differs from the electronic image, owing to differences in diffractive qualities of the two types of rays. Several methods of using the electron microscope in biology are considered and suggested.

R. J. Lebowich.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

Jan. 28, 1939

SAMUEL SANES, *Secretary*

CHORIOANGIOMA OF THE PLACENTA. LOUIS A. SIEGEL and EMERSON HOLLEY.

A white American housewife, 32 years of age, gravid for the second time, was admitted in labor to the Children's Hospital, June 4, 1938. Examination disclosed a right occipitoanterior presentation and position; by rectal examination the cervix was felt to be half dilated.

Two hours after admission the membranes were ruptured artificially; a live normal boy was delivered spontaneously. The placenta and membranes were expelled completely, following use of a modified Crede technic.

The placenta was of average normal size and shape, but situated on the fetal surface near the free margin was a mass the size of a hen's egg. It measured 9 by 6 by 4 cm. The tumor was well defined, solid and firm and could be readily enucleated from the surrounding placental tissue.

Microscopic examination of the tumor showed it to be composed of a sparse amount of connective tissue stroma and an abundance of capillary-sized spaces which were lined by endothelial cells of a rather typical nature, Many of the spaces were empty; others contained red blood cells. In still other spaces remnants of hemolyzed red cells were seen. The tumor was divided into lobules by trabeculae of somewhat compressed, chiefly collagenous connective tissue, and surrounding the tumor and separating it from the placenta was a zone of compact fibrous tissue which formed a pseudocapsule.

The histologic diagnosis was chorioangioma of the placenta.

This report will be published in full in the *American Journal of Obstetrics and Gynecology*.

THE DEVELOPMENT OF AGGLUTININS IN CHILDREN WITH DYSENTERY DUE TO SHIGELLA PARADYSENTERIAE FLEXNER. ERWIN NETER.

The serodiagnosis of infections due to Shigella paradysenteriae Flexner is considerably more difficult than that of typhoid fever or of dysentery caused by Shigella dysenteriae Shiga. In the first place, Shigella paradysenteriae Flexner comprises a number of antigenically different types, including Shigella paradysenteriae V, W, X, Y and Z of Andrewes and Inman and other types described by Kruse and his co-workers, Boyd, Sartorius and Reploh. Second, the titer of normal agglutinins for members of the Flexner group shows marked variation and may be as high as 1:500 and above. Third, it has been reported by some authors that specific agglutinins may fail to develop in patients with dysentery caused by the Flexner organism. Finally, patients suffering from infections with Shigella alkalescens (Andrewes) may acquire immune agglutinins for certain types of S. paradysenteriae Flexner because of common antigenic components present in both species (Neter, E., and Gilfillan, R. S.: *Am. J. Hyg.* **29**:67, 1939).

During the summer of 1938 23 patients with the clinical symptoms and signs of acute dysentery were admitted to the Children's Hospital. One of the patients showed marked signs of meningeal irritation, with rigidity of the neck, Kernig's

phenomenon, and other signs and resembled a patient with the type of bacillary dysentery which was described by Felsen (*Am. J. Path.* **12**:395, 1936). All the patients recovered. One patient had cystitis due to S. paradysenteriae (Neter, E., and Fisher, W. J.: *Urol. & Cutan. Rev.* **42**:810, 1938). Dysentery bacilli could be isolated from the feces of 17 of these 23 patients. The micro-organisms belonged to the Flexner group. They fermented dextrose and mannitol, with production of acid but not gas; they failed to attack lactose, sucrose, rhamnose, xylose and dulcitol. The dysentery bacilli isolated from 15 patients were of the W type of Andrewes and Inman; the micro-organisms isolated from the remaining 2 patients were of the Y type. Of the patients infected with S. paradysenteriae W, 8 were followed with respect to the development of specific agglutinins. Twenty-eight normal serums and serums of patients with diseases other than dysentery failed to agglutinate this particular type strain in titers above 1:25. In contradistinction, 7 of the 8 serums from patients with dysentery showed agglutinins in titers ranging from 1:80 to 1:1,280. In addition, the agglutination was manifest after only fifteen minutes to two hours' incubation at 55 C. and was considerably stronger than that obtained with normal serums. High titers of agglutinins were observed on the seventh to fifteenth day after the onset of the illness. In several cases serum specimens were tested during the first five days of illness and found to lack agglutinins for the strain of S. paradysenteriae which was isolated from the patients. In one particular case, for instance, the serum obtained on the third day of the disease had an agglutinin titer of less than 1:10, while the serum obtained on the seventh day of the illness strongly agglutinated the strain under investigation in a dilution of 1:80. One patient presenting the clinical signs of dysentery was never found to have dysentery bacilli in the feces; the development of specific agglutinins during the course of the illness in this particular patient gives strong evidence as to the cause of the disease, particularly since this child was 1 of 3 siblings in all of whom dysentery developed.

It may be concluded from these observations that in outbreaks of bacillary dysentery, as presented in this article, the examination of the serum of the patients for immune agglutinins for the homologous micro-organisms may support and supplement the bacteriologic findings.

PANCREATOGENOUS STEATORRHEA IN AN INFANT. S. SANES and RICHARD A. DOWNEY.

At birth a white boy weighed 6 pounds 15 ounces (3.147 Gm.). He was the first born. In the second week of life he began to have three to twelve stools per day. Some of these were loose and watery. Most were large, soft, yellow and foul. Fat in the stool was 51 per cent of the dry weight. Diets prescribed for their constipating effect brought no relief from the frequent large stools. When the boy was 3 months old, signs of a respiratory infection developed. The abdomen also became distended. Despite the fact that he took his feedings well, he remained the same weight as at birth until his death, at 7½ months of age.

The anatomic diagnosis was: pancreatogenous steatorrhea; marked marasmus; anemia; hyperplasia of the bone marrow; marked purulent bronchitis and bronchiolitis with bronchiectatic abscesses and chronic interstitial pneumonia and bronchopneumonia; atypical metaplasia of the epithelium of bronchioles; pulmonary emphysema; gross atresia of the cystic duct with glandular formations in the wall containing inspissated secretion; slight rickets; hyperemia with hemorrhage in the medulla of the adrenal; digested milklike content in the trachea and bronchi; hyperemia and hemosiderosis of the spleen; hemosiderosis of the liver.

Microscopically, the lobular structure of the pancreas could still be discerned. The acinar tissue was not well preserved. The ducts were dilated. They contained detritus, apparently secretion, desquamated cells and few neutrophils. Fat was demonstrated in the ductular content, particularly within large cells. There was distinct interlobular and intralobular inflammatory reaction, with infiltration of round cells, plasma cells, eosinophils and neutrophils and marked fibrosis. Hemosiderin was revealed free and in macrophages. The capillaries were dilated.

Several medium-sized arteries showed hyaline thickening of the internal elastic lamina. The walls of these vessels as well as of smaller arteries seemed somewhat thickened. Islets of Langerhans were present in good numbers. Some were hypertrophic. Bacteria were not found with special stains.

The observations in our case correspond to those summarized by Anderson and Blackfan in their recent publications. We interpret the lesion in the pancreas in the light of obstructive cirrhosis.

PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

Regular Meeting, Jan. 12, 1939

H. L. RATCLIFFE, *Secretary*

ALEUKEMIC PLASMA CELL LEUKEMIA WITH HEMORRHAGIC PHENOMENA. MITCHELL BERNSTEIN and DAVID FISHBACK.

Aleukemic plasma cell leukemia is rare. Its occurrence with hemorrhagic phenomena is still more unusual. Our patient, aged 52, complained of bleeding from the gums and marked weakness when he first came under observation, on Feb. 2, 1938. The bleeding from the gums began in November 1937 and occurred almost daily.

The striking features were: pallor, hemorrhages from the gums, petechiae in the buccal membrane, a soft apical systolic cardiac murmur, enlargement of the spleen to 8 cm. below the left costal margin, and a few petechiae over the thighs. Marked secondary anemia was found, the hemoglobin content being 27 per cent, the erythrocyte count 1,750,000 and the leukocyte count 3,500 per cubic millimeter, with 60 per cent neutrophils, 37 per cent lymphocytes and 3 per cent monocytes. The platelets averaged 110,000 per cubic millimeter. This blood picture did not change with subsequent examinations. The bleeding time was six and one-half minutes, and the coagulation time was three and one-half minutes. Chemical examination of the blood revealed hyperproteinemia, reversal of the serum albumin-globulin ratio and a low cholesterol content. An unusual feature of the blood was its peculiar tendency to gel on withdrawal from the patient's vein regardless of the addition of citrate or oxalate.

The urine contained albumin, hyaline casts and occasionally double refractile bodies. Routine studies of the gastrointestinal tract showed a normal condition. Roentgen studies of the skeleton revealed nothing of interest. The sternal bone marrow during life was said to be hypoplastic.

From February 2 on, the patient had repeated oozing hemorrhages from the gums and nose. On May 18 bilateral thrombosis of the retinal veins occurred, and weakness, pallor and splenic enlargement persisted. Slight enlargement of the liver was noted. Bronchopeumonia finally developed, and death occurred on June 22.

At necropsy the features of interest were: splenomegaly, marked myocardial degeneration, infarction of the left lung, confluent bronchopneumonia, nephrosis and nephrosclerosis, fatty metamorphosis of the liver, with slight congestion, and hyperemia of the brain. Histologic examination (Dr. R. P. Custer) showed various tissues, especially the spleen, bone marrow, lymph nodes, liver and adrenals, to contain large collections of plasma cells. Plasma cells were also noted within a small blood vessel.

DISSECTING ANEURYSMS SIMULATING SYPHILITIC HEART DISEASE, WITH AORTIC REGURGITATION. E. O. ANDERSON and B. A. GOULEY.

A study of autopsy records at the Philadelphia General Hospital during the past ten years has disclosed a total of 40 cases of dissecting aneurysm—or an incidence of 1 in 400. In 16 of the 40 cases the classic signs and symptoms of rapid dissection were presented, with death due to external massive hemorrhage. In 6 of the 40 cases there were signs and symptoms of aortic regurgitation. Death was due not to rupture of the aneurysm and extravasation of blood but to long-standing heart failure. At autopsy the cross section of the aorta usually presented an onion peel appearance. The dissection of the media frequently extended the entire length of the aorta, from the root to or beyond the bifurcation of the iliac arteries. In 2 cases the dissection remained within the confines of the media, there having been neither internal or external rupture. In the other cases the dissection communicated with the true aortic lumen both proximally and distally, producing a double barrel channel. There was no separation of the commissures, but the aortic ring was dilated, and the central portions of the free margins of the aortic cusps were moderately rolled and thickened so as apparently to prevent adequate approximation. The heart was invariably hypertrophied. In 3 additional, similar cases, the dissection had not extended proximally to the root of the aorta or had not existed long enough to cause signs and symptoms of aortic regurgitation, and these instances were therefore excluded from this series.

The clinical diagnosis in the majority of the cases was syphilitic heart disease with aortic regurgitation. Even at autopsy in 4 of the 6 cases the gross anatomic diagnosis was syphilitic aortitis. However, in no case could syphilitic aortitis or any other syphilitic entity be substantiated by microscopic examination.

RECURRENT BENIGN PROSTATIC HYPERTROPHY ASSOCIATED WITH CARCINOMA. W. P. JENNINGS.

According to available information, the incidence of recurrent benign hypertrophy of the prostate is approximately 2 per cent. From a brief review of reports it appears that the development of carcinoma of the prostate in association with recurrent hypertrophy is much less frequent. Two examples of the latter condition are presented here. The ages at which both the first and the second phase of the disease occurred are within the expectable range. The patients were recently admitted to the Presbyterian Hospital, to the services of Dr. George C. Griffith and Dr. Joseph C. Birdsall, respectively.

CASE 1.—A white man, 71 years old, was admitted to the hospital Nov. 9, 1938, because of hematuria of five weeks' duration. In June 1933 an enlarged prostate had been removed, which showed adenofibromatous hypertrophy and chronic prostatitis. The patient's convalescence was uneventful. In June 1937 he noted increasing constipation. From July 1938 to the date of admission he lost 50 pounds (22.7 Kg.) in weight and experienced weakness of the arms and legs, dyspnea and intercostal neuralgia. In October 1938 hematuria was noted, and the testes were swollen and painful. Proctoscopic and cystoscopic examination apparently were not informative. The blood pressure was elevated. There was marked tenderness over the left side of the abdomen, as well as slight tenderness over the right, chiefly over an enlarged liver. The scrotum was swollen and inflamed, with edema of the scrotum and on the left side, and the left testis was enlarged and soft. The rectal sphincter was spastic, and a large hard mass could be palpated on the anterior rectal wall. The urine contained a trace of albumin and numerous leukocytes. The blood count was normal. The patient died on November 22. At autopsy the immediate cause of death was demonstrated to be a coronary occlusion with infarction of the interventricular septum. The bladder was trabeculated. The urethral mucosal surface was smooth and white, with two firm white sessile nodules, 3 and 2 cm. in diameter, respectively, bulging from the lateral surfaces of the otherwise smooth prostatic bed, producing no obstruction. Periurethrally in the prostatic region was a mass of firm white tissue 6 by 5 by 3 by 2 cm.

within which were numerous punctate yellow areas and minute, extremely hard white elevations. The main portion of this extended from the urethra to the rectum. The testes and penis were apparently normal. Microscopically, the superficial tissues in the prostatic urethra contained a mass of prostatic elements showing benign hypertrophy. A few hyperplastic ingrowths were present. However, the underlying muscular coats and periurethral tissues were infiltrated by neoplastic cells, among which few mitotic figures were seen. Chronic inflammatory changes were associated with this process. Distal to the prostatic tissue, the urethral mucosa was ulcerated, and the superficial as well as the deep fasciae were infiltrated with tumor cells. The diagnosis was primary adenocarcinoma of the prostate, grade 3, and recurrent benign hypertrophy. Metastases were not found.

CASE 2.—A white man aged 82 years was admitted to the hospital on Nov. 29, 1938, because of increasing frequency of urination of two weeks' duration, resulting in acute retention on the morning of admission. Transient hematuria was noted in October 1938. In September 1917 an enlarged and indurated prostate had been removed. Convalescence was uneventful. At the present admission the results of physical examination were essentially negative; there was, however, slight cardiac enlargement, and the prostate was large, irregular and hard. Cystoscopic examination revealed marked trabecular hypertrophy and what were apparently diverticula; there was a prominent anterior notch with protruding intravesical, lateral and median lobes. On December 14 a large prostatic mass, 7 by 5 by 3 cm., and four smaller masses, each 1 cm. in diameter, collectively weighing 95 Gm., were removed. The prostatic tissues were well encapsulated, had a honeycomb appearance and were composed of multiple yellowish white masses of varied size in a soft fibrous matrix. The gross impression was recurrent benign hypertrophy. Microscopically, sections through seven portions of the gland were examined, all of which showed bulky, often greatly dilated glands lined by hyperplastic columnar epithelium. In several areas, however, the epithelium assumed a distinct neoplastic character and was definitely invasive. Tumor cells were noted in a dilated lymphatic channel. The diagnosis was recurrent benign prostatic hypertrophy and adenocarcinoma of the prostate.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF THE CHICK EMBRYO. LAWRENCE E. W. SMITH.

The effect of changes in temperature on the development of the chick embryo was found to be profound in a series of over 700 instances. A critical temperature lying somewhere between 90 and 95 F. exists at which not only delay in development was noted but also failure of cell growth, differentiation and organization. The significant fact was observed that the first forty-eight to ninety-six hours were the most important. Eggs which at the beginning of their development were subjected to a temperature of 90 F. for forty-eight or more hours and then allowed to complete their incubation at a normal temperature (102-103 F.) showed 100 per cent malformations. These ranged from minor defects of the extremities to actual monstrosity. Ectopia cordis, eventration, sirens and even double-headed monsters were obtained with absolute regularity. Temperatures well below this critical level resulted merely in delayed maturation. Temperatures above 105 or 106 F. caused rapid distintegration of the embryo.

These results point toward a basic biologic principle in relation to cell growth. Its possible application as a therapeutic adjunct in the treatment of inoperable, advanced cancer, dependent on the idea of a critical level of temperature for young, actively growing tumor cells, is suggested. Preliminary clinical observations in a small group of cases seem to bear out the soundness of this idea.

"SILENT," SO-CALLED PRIMARY TUBERCULOSIS OF THE SPLEEN. HERBERT FOX.

Three cases are reported in which so-called primary tuberculosis of the spleen was discovered by splenectomy. The condition is called primary because no other

locality of disease could be unexceptionally diagnosed. The term "silent" is suggested because only the splenomegaly called attention to the condition. Tubercle bacilli were sought for by all the accepted methods without success, and this has been the experience of others. Tuberculosis of the spleen has been found associated with purpura, pernicious anemia, hemolytic icterus, leukemia, polycythemia and Banti's syndrome. The silent form is usually fibromiliary; the caseous form is associated with more easily discoverable tuberculosis. The tuberculin test is unreliable. This chronic fibromiliary form seems never to have been diagnosed before operation.

The article will be published in full in the *American Journal of Clinical Pathology*.

BAXTER L. CRAWFORD, *President*

Regular Meeting, Feb. 9, 1939

H. L. RATCLIFFE, *Secretary*

MURAL ENDOCARDITIS FOLLOWING PULMONARY SUPPURATION. A. TRASOFF and D. R. MERANZE.

A woman aged 47 years became suddenly ill with symptoms and signs suggestive of acute pancreatitis. These continued for two weeks, and then the condition followed a more septic and toxic course.

The physical signs were those of an infection of the upper respiratory tract and pneumonitis of the lower lobe of the right lung, with marked abdominal distention and epigastric tenderness. The blood studies all gave negative results except for anemia. The urine showed a trace of albumin.

Within two weeks after the onset a large spleen was palpable, and examinations of the blood, urine and feces for typhoid bacilli and brucellas revealed none. A roentgenogram of the lungs revealed marked congestion but no pneumonia. The disease progressed rapidly, the temperature becoming more septic; finally meningeal involvement became apparent. The patient died before a lumbar puncture could be obtained.

On postmortem examination were found: a large spleen, which contained a hemorrhagic cyst; a mural thrombus in the left auricle of the heart; a large abscess in the upper lobe of the left lung and smaller abscesses in the upper lobe of the right lung with unresolved pneumonia. Abscesses were also found in the liver, kidney, perirenal fat tissue and brain, from which a type III pneumococcus was isolated. The pancreas showed definite evidence of acute interstitial pancreatitis, as noted by marked edema and infiltration.

BENIGN ENLARGEMENT OF THE PROSTATE. ROBERT A. MOORE.

Benign enlargement or benign hypertrophy of the prostate occurs commonly in the presenile and senile periods of life. In postmortem examinations there is found a progressive increase in the incidence with increase in age, so that men in the ninth decade show an incidence of 75 per cent. In clinical examinations the greatest incidence is found in persons in the seventh decade of life, with fewer cases in both younger and older persons. The disease also shows varying incidence in some races and is more frequent in married than single men.

The earliest lesion is found in the periurethral tissue and consists of focal hyperplasia of the stroma. If glands are in immediate contact with the foci in the stroma, there is simultaneous hyperplasia of the epithelium. Only that part of the prostate is involved in which the ducts empty into the urethra above the lower end of the verumontanum. This part of the prostate is represented in the female pseudohermaphrodite. The prostate thus consists of two parts, a male part below

and an ambisexual part above the verumontanum. The same separation can be established in a study of the prostate of a newborn infant which has been exposed to a large amount of estrogen in the mother's blood.

On the basis of these facts and others it seems probable that the estrogenic hormone in the male organism plays a role in the genesis of benign enlargement. My own studies for the past two years have been directed toward development and proof of this working hypothesis.

It is well known that androgens will neutralize the effect of estrogens if injected in a ratio of about 50 to 1. It is also known that there is a gradual decrease in the urinary excretion of androgens with increasing age. It follows that if the amount of effective androgen decreased more than the estrogen during presenility the prostate would be exposed to unneutralized estrogen. In an analysis of 60 specimens of urine from patients with and without benign enlargement there is evidence to support this assumption.

Studies of the morphologic aspects and hormone content of the pituitary gland in benign hypertrophy eliminate this organ as the primary seat of the disease. The absence of the disease in true eunuchs lends strong support to a theory that the most probable cause is a disturbance in the hormone secretion of the testes.

The most satisfactory working hypothesis is: Benign enlargement of the prostate is a disease of presenility due to the altered and irregular differential secretion of androgen and estrogen during this period of life.

Book Reviews

La maladie D'Aujeszky. P. Remlinger, directeur de l'Institut Pasteur de Tanger et J. Bailly, chef de service a l'Institut Pasteur de Tanger. Paper. Pp. 204, with 16 illustrations. Price 45 francs. Paris: Masson & Cie, 1938.

This is the only monograph on a disease known under several names, "infectious bulbar paralysis," "mad itch," "pseudorabies," but known best under the name of its discoverer, who described it in 1903, in Hungary, as a previously unknown disease in dogs, cats and cattle. In 1912 the disease was observed in Brazil, later in various European countries, then in Africa and Asia, and in 1930 it was found by Shope in the state of Iowa. A biographic introduction deals with the life of the discoverer, Aládar Aujeszky, 1869-1933. Then follow chapters on the history of the recognition of the disease all over the world, the symptoms it gives rise to in the different species and the complicated epidemiologic problems which it presents. While the disease has been found in only a limited number of animals, all of them mammals, it can be reproduced experimentally in a large number of species, among them all the usual warm-blooded laboratory animals, and by practically all the known routes of infection. Important observations on the experimental aspects of the disease are found in the third chapter. In man the disease has been observed in a few isolated instances, and its course was mild. The peculiarity of most of the affected animals that they die of the disease at night is the subject of a special chapter. The experimental tranmission of the disease, the characteristic posture of the dead animals, which resemble stuffed museum specimens, the scratch lesions, the self-inflicted mutilations due to extreme itching, the secondary infections, the rather insignificant changes in the internal organs, the inflammatory lesions in the central nervous system and the changes in the urine and blood are described in chapter 6. The cause of the disease, a filtrable virus, is present in practically all tissues of the body and absent from most of the secretions and excretions. The nature of the virus and its relation to the virus of rabies and of herpes are considered. The diagnosis and the treatment, what there is of it, are discussed, and good reasons are presented in favor of using the experimental forms of the disease for teaching of virus diseases in medical schools. An extensive bibliography, which includes thirty publications by the authors, concludes the monograph. This work ought to be of great interest to veterinarians and to students of virus diseases.

The Biology of Bacteria. An Introduction to General Microbiology. Second edition. Arthur T. Henrici, Professor of Bacteriology, University of Minnesota. Pp. xiii + 479 and index. Price $3.60. Boston: D. C. Heath & Co., 1939.

That a second edition of Dr. Henrici's text on general microbiology should appear is in itself a guaranty of merit; that it should appear so soon after publication of the first edition indicates not only a continuing growth in the subject matter but also a realization that the science of bacteriology can be well served only by making its discoveries and the newer interpretations based thereon available to the present day student. Textbooks in such an active field must inevitably be somewhat behind the frontier, but surely both the science and the scientist deserve a frequent sharpening of their tools. This is provided in this treatment of the basic phases of bacteriology. Individual chapters have been revised, and additional chapters have been introduced, to the end that a more complete and a broader point of view may be presented to the student. The text meets the purpose admirably.

The author is to be commended for acting on the very evident fact that today no single course in bacteriology can meet all needs, no single author or teacher of bacteriology can be competent in all fields and that no single textbook of bacteriology adapted to practical use by the student can treat authoritatively all phases of the subject. An introductory course based on this text will provide an excellent point of departure for advanced study in many fields.

Books Received

RECENT ADVANCES IN FORENSIC MEDICINE. Sidney Smith, M.S., F.R.C.P., D.P.H., Regius Professor of Forensic Medicine, University of Edinburgh; and John Glaister, M.D., D.Sc., J.P., of the Inner Temple, Barrister-at-Law, Regius Professor of Forensic Medicine, University of Glasgow. Second edition. Cloth. Pp. 264, with 85 illustrations. Price $4.50. Philadelphia: P. Blakiston's Son & Co., 1939.

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, B.A., M.A., M.D. Volume XXX, 1938. Cloth. Pp. 1,065, with 149 illustrations. Price $11.50. Philadelphia and London: W. B. Saunders Company, 1939.

THE HARVEY LECTURES. Delivered under the Auspices of the Harvey Society of New York, 1938-1939. Under the Patronage of the New York Academy of Medicine. Dr. Guy F. Marrian, Dr. A. Ashley Weech, Dr. Eugene F. du Bois, Dr. Edwin J. Cohn, Dr. Edwards A. Park, Dr. K. Linderstrøm-Lang, Dr. C. H. Danforth, Dr. Albert Szent-Györgyi. Series XXXIV. Cloth. Pp. 279, with illustrations. Price $4. Baltimore: Williams & Wilkins Company, 1939.

BLOOD GROUPS IN AFRICA. Ronald Elsdon-Dew, M.D. Publications of the South African Institute for Medical Research, Edited by the Director. No. XLIV (Vol. IX, pp. 29-94). Paper. Johannesburg, South Africa: The South African Institute for Medical Research, 1939.

CORRECTION

In the review by Drs. Weil, Gall and Wieder entitled "Progress in the Study of the Typhoid Bacillus," in the July issue of the ARCHIVES (**28**:71, 1939), reference was made on page 78 to a statement by Drs. Delafield and Smith regarding the effect of somatic antigen on the oxygen uptake of organs. The June issue of the *British Journal of Experimental Pathology* (**20**:216, 1939), however, contains a later report by these authors, stating that the effect was due to some material which was present in the trypsin used in the preparation of the antigen, and not to the antigen itself.